UNIVERSITY OF TASMANIA

Surviving under the Antarctic sea ice - a study of the feeding ecology of Antarctic krill

by

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"She's a Kriller Queen
Labcoat and formalin
Killer shrimps with a laser beams
Guaranteed to blow your mind"

– Dr. Hugo Jones

Abstract

The seasonal advance and retreat of sea ice is a key feature of the Southern Ocean. Antarctic sea ice covers approximately 4 million km^2 during minimum extent in February/March and grows to 19 million km^2 during maximum extent in September/October. This sea ice zone harbours a wide diversity of biota and supports large populations of unique Antarctic organisms. During the satellite era, Antarctic sea ice has shown strong regional changes in its extent, duration, and the timing of the annual advance and retreat. Improving our understanding of the relationships between sea ice and ice-associated animals will fill a crucial knowledge gap, which will facilitate the conservation and management of the Southern Ocean ecosystems and resources.

This thesis compiles three studies focusing on the feeding ecology of Antarctic ice-associated zooplankton during the winter-spring transition. This work concentrates on Antarctic krill (*Euphausia superba*), a key species with ecological and commercial significance in Southern Ocean ecosystems.

Morphology defines an animal's feeding capability. The morphological changes through ontogeny of a species thus are not only important taxonomic information, but also crucial for our understanding regarding its feeding ecology. In Chapter 2, I describe the morphological changes of *E. superba* through its development from an egg to juvenile. This is the first study since 1936 that fully describes the morphological features of this species, in which I update the existent knowledge with details and high-resolution photographs. Intermediate larval stages are identified and these morphological characteristics are related to the overwintering survival strategies for this species.

The food that animals actually obtain from the environment can be investigated with either gut content or biochemical analyses. To determine the dietary preferences and trophic relationships of major zooplankton species (Chapter 3), I use stable isotope analyses ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to compare samples collected from East Antarctic pack ice zone during two winter-spring transitions (2007 and 2012). Interannual dietary differences are determined for larval *E. superba*, suggesting feeding plasticity, which enables this species to adapt to changing environmental conditions. Larval *E. superba* are primarily herbivorous while utilising sea-ice biota, and consume a more heterotrophic diet when feeding from the water column. In contrast, post-larval *E. superba*, and the omnivorous krill *Thysanoessa macrura* consume a mixed diet from both the water column and the sea ice. The pteropod *Limacina helicina*, small copepods in the genus *Oithona* spp., ostracods and amphipods rely heavily on sea-ice biota according to their carbon isotope ratios. Large copepods and chaetognaths consume a water column-based diet during the winter. The comparison of isotopic profiles between years suggests that ice-associated

zooplankton gain access to sea-ice biota more easily under warm and permeable ice than under cold ice.

Our ability to construct food web models for sea-ice ecosystems are restricted by limited real data and inadequate understanding regarding the system structure. A qualitative modelling approach is applied in Chapter 4 to explore how the increasing model complexity affects model predictions, and to identify key variables and interactions governing krill dynamics in this system. A series of qualitative network models are constructed to represent different theories of krill winter feeding, and winter conditions are simulated by applying a perturbation of an increase in sea-ice algae and a decrease in pelagic phytoplankton concurrently. Results demonstrate the importance of including developmental-stage-specific information during the construction of food web models. In addition, model outcomes suggest that the coupling between primary production (either sea-ice or pelagic) and protozoan production should be investigated in future empirical studies as it is a key process affecting model predicted krill responses. This study also demonstrates the usefulness of qualitative network modelling in hypotheses testing regarding ecological processes.

This thesis has contributed to a better understanding of feeding ecology of Antarctic krill and other under-ice zooplankton during the winter-spring transition period. My results suggest that future studies regarding feeding ecology of ice-associated zooplankton should use combined approaches. The use of qualitative modelling could help evaluate assumptions based on limited data, which is advantageous for identifying critical ecological processes and pinpointing the empirical observations that are needed. In addition, advanced technologies, such as high-resolution underwater camera and underwater vehicles, will allow us to obtain habitat-structure information and detailed in situ behavioural observations, which should also be evaluated and considered in future feeding studies.

Statement of Co-authorship

Dr. Patti Virtue (IMAS) and Dr. Kerrie M. Swadling (IMAS, ACE CRC) provided comments on the general sections of this thesis (**Chapter 1 and 5**). Dr. Klaus Meiners (AAD, ACE CRC), Dr. So Kawaguchi (AAD, ACE CRC), and Dr. Simon Jarman (AAD) provided comments on the introduction (**Chapter 1**).

Chapter 2 - 4 of this thesis have been prepared as manuscripts for submission to peer-reviewed journals. I was the lead author for each of the manuscripts and responsible for the design and implementation of the research, data analysis, interpretation of results and manuscripts preparation. The co-authors contributed to either the collection of data, some experimental design, and played a role in supervision of my PhD and revisions of manuscripts for publication. Contributions of co-authors are outlined below:

Chapter 2: Dr. Patti Virtue (IMAS), Dr. Kerrie M. Swadling (IMAS, ACE CRC), and Dr. So Kawaguchi (AAD, ACE CRC) contributed to the development of the ideas, and provided comments on the manuscript.

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Chapter 3: Dr. Patti Virtue (IMAS) provided assistance during sample collection. All contributing authors contributed to the development of the ideas, and provided comments on the manuscript.

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Chapter 4: Dr. Jess Melbourne-Thomas (ACE CRC, AAD), Dr. Martin Marzloff (IMAS), Dr. Patti Virtue (IMAS), Dr. So Kawaguchi (AAD, ACE CRC), and Dr. Klaus Meiners (AAD, ACE CRC) provided advice on model development and scenario testing. Dr. Jess Melbourne-Thomas and Dr. Martin Marzloff provided advice on interpretation of model results. All contributing authors provided comments on the manuscript.

Manuscript has been prepared to submit to PloS One.

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Dr. Patti Virtue, *Primary Supervisor*

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Dedicated to Henrietta, and hundreds of krill analysed in this study

Chapter 1

Introduction

1.1 Sea ice

Southern Ocean sea-ice cover ranges from 4 million km^2 during minimum in the summer to 19 million km^2 during maximum in the winter/spring. The seasonal formation and melting of sea ice characterises this region as one of the most extensive and dynamic habitats on the planet (Brierley and Thomas, 2002; Arrigo, 2014). Sea ice serves as a refuge, a foraging ground, a resting platform, and a breeding and nursery ground for numerous planktonic organisms as well as many ice-dependent predators in the Southern Ocean (Knox, 2007; Flores, 2009; Thomas and Dieckmann, 2010; Swadling, 2014). The distribution, structure and extent of sea ice are highly sensitive to climatic conditions (Lubin and Massom, 2007; Flores, 2009; Massom et al., 2013). Significant changes in sea-ice conditions have already occurred in Arctic ocean (Stroeve et al., 2007), and these changes in ice extent, age, and duration have greatly impacted on Arctic ice associated ecosystems (Moline et al., 2008). Rapid changes are also taking place in specific regions of the Southern Ocean, which creates uncertainty about the future for sea-ice based ecosystems (Constable et al., 2014).

Sea-ice extent in Arctic has declined by more than 20% since 1979 (Alexander et al., 2013). In addition, changes in the timing of sea-ice advance and retreat have resulted in extended ice-free seasons, and ice-free summers are expected within the next 30 years (Serreze et al., 2007). These changes in sea-ice extent and seasonality have direct and indirect ecological impacts. The reduction of sea ice and warming temperature have directly reduced biodiversity, and altered the composition of sea-ice algal communities (Melnikov, 2009). Indirectly, changes in sea-ice conditions have induced hydrographical alterations, resulting in sub-Arctic species shifting to higher latitudes. This introduces

potential competitive pressure on Arctic species (Moline et al., 2008). Reduction in sea-ice habitats also severely impacts ice-dependent mammals. For example, polar bears have suffered from habitat loss and an increase in the length of the ice-free season, which are threats to their long-term survival (Stirling and Derocher, 2012). Changes in Arctic sea-ice extent and seasonality are likely to challenge the survival of many ice-associated species with flow-on effects on Arctic food web structures (Marz, 2010).

Unlike the constant decline in Arctic sea ice, sea ice around the Antarctic continent has shown a more complex trend over the last three decades (Massom et al., 2013), making it more difficult to understand and estimate potential ecological impacts. The overall Antarctic sea-ice extent has increased slightly over the last 30 years and reached its maximum recorded extent in 2014, covering 20 million km^2 (Ramsayer, 2014). However, this trend masks fast and large Antarctic sea ice changes on regional scales, with rapid increase in sea ice extent in the Ross Sea (Turner and Overland, 2009) and decrease in the southern Bellingshausen and eastern Amundsen seas (Massom and Stammerjohn, 2010; Stammerjohn et al., 2012). Sea-ice duration and seasonality also show considerable regional differences. In the Antarctic Peninsula and Bellingshausen Sea region, the sea-ice season has become three months shorter, whereas in the Ross Sea region, it has increased by nearly three months (Stammerjohn et al., 2012). In contrast to these changes seen in West Antarctica, sea ice across East Antarctica has shown a less profound but more complex trend in extent and seasonality (Massom et al., 2013). Both strong positive and negative trends in extent and seasonality have occurred in small local scales.

Changes in sea ice, especially in the timing of sea-ice advance and retreat could lead to changes in habitat, ecosystem dynamics and biogeochemical cycling (Ducklow et al., 2007, 2012). Very few animal species in Antarctica have been studied long enough to fully assess the ecosystem responses to sea ice changes (Nicol et al., 2011; Trivelpiece et al., 2011). To determine the impact of sea-ice changes on Antarctic oceanic ecosystems, a better understanding of the role of sea ice within Antarctic food webs is urgently required (Flores et al., 2012a). Improved understanding of the relationship between the sea ice and ice-associated animals is needed to support better ecosystem based management strategies for the conservation of Southern Ocean ecosystems (Constable et al., 2014).

When sea ice forms, ice crystals scavenge a range of algae, bacteria, protists and invertebrates from the water column (Garrison et al., 1983; Garrison, 1991b). Sea ice is permeated with brine channels and pores that provide incorporated biological materials a platform upon which they can remain suspended in the upper ocean. Some of the incorporated organisms flourish as the ice ages over the year (Thomas and Dieckmann, 2010). As a result, algal biomass in the ice is considerably greater than in the underlying water column during autumn and winter, and through early spring (Quetin and Ross,

2009; Thomas and Dieckmann, 2010). However, sea ice demonstrates a diverse physical feature even at a very small scale, which subsequently affects ice-algal concentration at a local level. Therefore, algal biomass concentrates in small areas of sea-ice rather than distributed evenly. This concentrated sea-ice biota plays a critical role in the Southern Ocean ecosystem by providing an alternative carbon source for zooplankton grazers (Thomas and Dieckmann, 2010). Furthermore, winds and currents reshape the ice, creating an over-rafted habitat consisting of gaps and caves within the pack, which appears to be an important winter refuge for a variety of zooplankton species (e.g. *Euphausia superba*) (Brierley and Thomas, 2002; Meyer et al., 2009; Massom and Stammerjohn, 2010).

1.2 Zooplankton community and sea ice

Ice-associated zooplankton form a key component of the diet of many top predators, representing an essential link between sea-ice primary production and higher trophic levels in the Southern Ocean (Thomas and Dieckmann, 2010; Flores et al., 2011). Knowledge of trophic interactions between sea-ice biota and zooplankton is critical for estimating their roles in energy flow and carbon flux through Southern Ocean ecosystems (Dubischar and Bathmann, 1997; Buitenhuis et al., 2010; Flores et al., 2011; Atkinson et al., 2012c). Despite its importance, to date, the diet of zooplankton under the sea-ice cover is still poorly understood due to limited sampling, both spatially and seasonally. Studies of sea ice and associated zooplankton in the Southern Ocean are limited and mostly restricted to shelf waters and land-fast ice (e.g. Tanimura et al., 1996; Swadling et al., 1997, 2000; Tanimura et al., 2008). Biological interactions within the pack-ice zone remain relatively unknown because of the logistical difficulties of sampling in the sea ice during ice-covered seasons (Brierley and Thomas, 2002; Arndt and Swadling, 2006; Hunt et al., 2011; Swadling, 2014).

1.2.1 Zooplankton overwintering strategies

Scarcity of food is the major challenge for zooplankton winter survival. Earlier hypotheses of zooplankton overwintering strategies included 1) undergoing diapause at depth; 2) switching diet to omnivorous feeding or detrital food sources; and 3) consuming the usual diet. Additionally, 4) body shrinkage has been posed as an overwintering strategy for Antarctic krill (*Euphausia superba*, Ikeda and Dixon, 1982). 5) Utilising food from the sea-ice habitat. More recent studies have emphasised that only few herbivorous species, such as *Calanoides acutus*, undertake a true winter diapause in the Southern Ocean (Schnack-Schiel and Hagen, 1995; Pasternak and Schnack-Schiel, 2001), while

most other species have a broader diet, and extend their feeding period into winter months, or overwinter within or under the sea ice (Conover and Huntley, 1991; Atkinson, 1998; Atkinson et al., 2002; O'Brien et al., 2011; Swadling, 2014).

From autumn through to early spring, concentrated algal biomass within pack ice is several orders of magnitude higher than in the water column (Quetin and Ross, 2009), which serves as the primary carbon source for zooplankton inhabiting the ice or in the underlying water column (Daly, 1990; Conover and Huntley, 1991; Quetin and Ross, 2009). Copepods dominate within the sea-ice matrix, and euphausiids often dominate at the ice-water interface under the sea ice (Arndt and Swadling, 2006; Swadling, 2014). Several copepod species (e.g. *Stephos longipes* and *Paralabidocera antarctica*) are referred to as ice specialists, spending part of or their entire life cycle within the sea-ice matrix (Hoshiai et al., 1987; Schnack-Schiel et al., 1995; Tanimura et al., 1996). A few small copepod species (e.g. *Oithona simillis* and *Oncaea curvata*) are abundant under the ice, and are sometimes found inhabiting the ice matrix itself, suggesting they have at least a limited association with the ice (Swadling, 2014). On the other hand, there is no current evidence that any large copepods live within the sea ice. Of the five most abundant and most commonly studied copepod species, *Calanus propinquus* and *Metridia gerlachei* have been sampled occasionally from the sea ice (Hoshiai and Tanimura, 1986). In particular, *C. propinquus* have been observed in the top 50 m of the water column under the sea ice (Hopkins and Torres, 1989; Bathmann et al., 1993; Hunt et al., 2011), and it has been suggested that this species utilises ice algae (Bathmann et al., 1993; Schnack-Schiel et al., 1997). However, the extent to which *C. propinquus* and smaller copepods interact with sea ice is still under investigation (Atkinson, 1998; Atkinson et al., 2012c; Swadling, 2014).

Antarctic krill live in close contact with the underside of the pack ice, and often dominate the community in the underlying water column. Scuba diving observations have documented aggregations of larval krill living in caves formed by over-rafting pack ice (Frazer et al., 2002; Meyer et al., 2009; Flores et al., 2012b), and them directly grazing on sea-ice algae from the bottom section of the sea ice (Daly, 1990; Meyer et al., 2009). Although under-ice habitat is considered critical for larval krill survival over the winter, the contribution of ice biota to their winter diet is still unclear. Furthermore, interaction between krill and other species under the ice remains unclear.

With the development of new technology, recent studies, using under water cameras (Watanabe et al., 2006) and Surface and Under Ice Trawls (SUIT, Flores et al., 2011) have discovered an under ice community that is far more diverse than previously known. Alongside Antarctic krill, a range of abundant species was collected from the ice-water

interface, such as amphipods, pteropods, and chaetognaths (Flores et al., 2011). However, we know little about how non-euphausiid species utilise the sea-ice habitat. For many of these species, the literature on their winter feeding is often conflicting. This is likely to be a reflection of within-species variability in overwintering strategies at different geographic locations, which has been increasingly recognised for *E. superba* in recent studies (Schmidt et al., 2014). Therefore, to gain a general picture of how sea-ice habitat could benefit zooplankton species in the winter, more research into the trophic interactions within the sea-ice community is required, in particular for regions with limited observations.

1.2.2 Antarctic krill *Euphausia superba*

Among all the zooplankton species, Antarctic krill, *Euphausia superba*, has received the most attention because of its important ecological role and the increasing commercial interest. *Euphausia superba* is considered a keystone species in Southern Ocean food webs as it is the main prey for many predators, such as baleen whales, seals, penguins, flying seabirds and fish (Deagle et al., 2007, 2008; Jarman et al., 2013). Krill is also an effective grazer on primary production. Adult krill are larger in size compared to other zooplankton species in the system, which makes them more effective with respect to biogeochemical flux and energy transfer (Hill et al., 2012; Ballerini et al., 2014). Since the mid-1970s, *E. superba* has been the target of a major fishery around Antarctica (Nicol and Endo, 1997; Nicol et al., 2011). With a fast growing krill fishery industry in the Southern Ocean, it is important to implement ecosystem-based management practices in order to prevent fishery induced irreversible changes in krill productivity or ecological relationships within Southern Ocean food webs (Hill, 2013).

Krill studies before the 1980s were predominantly driven by commercial interests and concentrated on investigating krill distribution, and estimating krill abundance and biomass (Atkinson et al., 2012a; Meyer, 2012). Studies on understanding overwintering strategies in krill emerged with growing research interest in the krill life cycle (Quetin and Ross, 1991). A range of both non-feeding and feeding overwintering strategies was suggested.

Antarctic krill undergo a complex life history comprised of 12 larval stages (Fraser, 1936; Marr, 1962; Kirkwood, 1982). Adult female krill that have been fuelled by the phytoplankton bloom in the spring spawn in the summer in surface waters. The embryos sink to the deeper ocean and hatch at depth (Quetin and Ross, 1984). Larval krill reach the surface water and begin feeding at the Calyptopis I stage. They spend the first 5 to 6 months developing through different larval stages and overwinter in a furcilia larval form

(Quetin and Ross, 2009). Critical periods for larval krill survival are during their first feeding stage, and during their first winter (Quetin and Ross, 1984; Ross and Quetin, 1988, 1989, 1991). Larval krill usually contain very low lipid content, and therefore need to feed throughout the year (Quetin and Ross, 1991, 2009; O'Brien et al., 2011). Juvenile krill and larvae, were similarly reported to be feeding through winter (Kawaguchi et al., 1986; Daly, 1990; Hopkins et al., 1993a; Stübing and Hagen, 2003). In contrast to larval krill, adult krill accumulate enough lipid and are able to tolerate prolonged starvation. Several non-feeding strategies, such as reducing metabolism (Atkinson et al., 2002), utilising lipid reserves (Hagen et al., 1996, 2001), and body shrinkage (Ikeda and Dixon, 1982; Virtue et al., 1996), have been documented for adult krill.

The literature on adult krill feeding during winter is still conflicting (Table 1.1). Atkinson et al. (2002) observed a substantial decrease in feeding rates in the winter in the Lazarev Sea, which suggested adult krill mainly relied on lipid reserves while feeding opportunistically. In contrast, other studies have shown krill feeding on copepods in the west Antarctic Peninsula (Huntley et al., 1994), on benthic materials in the East Antarctic near the Japanese Syowa Station (Kawaguchi et al., 1986), and on sea-ice biota in the Weddell Sea (Marschall, 1988). These various overwintering strategies reflect plasticity in krill behaviour and physiology during winter. A recent study by Schmidt et al. (2014) demonstrated that this variability is largely due to varying environmental conditions (i.e. sea-ice cover) in different geographic locations. Krill have proved difficult to catch in the winter due to the fact that they are strong swimmers and can escape current sampling devices. Although there are no direct observations of adult krill aggregating under the ice (Brierley et al., 2002), at this stage it is premature to conclude that they do not feed from sea ice.

TABLE 1.1: Summary of studies of Antarctic krill (*Euphausia superba*) feeding in the Southern Ocean during phytoplankton-depleted seasons. WAP = West Antarctic Peninsula; EA = East Antarctica; MIZ = Marginal Ice Zone. DNA = genetic-based methods; FA = Fatty acids and lipid; GF = Gut Fluorescence; I = Incubation experiment; M = Microscopy; Mo = Morphology description; O = Observation (laboratory or in situ); SI = Stable Isotope analysis.

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M	WAP	Mar-Apr, 1983	Mainly diatoms			No	Hopkins (1985)	
M	EA	May-Nov, 1984		Colour of stomach contents: May to Aug, yellowish; Oct, green		Fast ice	Kawaguchi et al. (1986)	Stomach colours indicate diet. Yellow, brown - detritus, green - algae
O	Weddell Sea	Jul-Sep, Oct-Dec, 1986	ROV: krill aggregate under pressure ridges. Lab: krill are able to graze algae from the bottom of ice			Pack ice	Marschall (1988)	ROV survey and lab observations
M	Weddell Sea	Mar, 1986	Phytoplankton 48%; protozoan 20%; metazoan 32%		Calyptopis: phytoplankton 97%	MIZ	Hopkins and Torres (1989)	AMERIEZ program

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Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M, GF	Scotia Sea	Jun-Aug, 1988		Omnivorous feeding	full guts: diatom and protozoan	MIZ	Daly (1990)	AMERIEZ program. Juvenile and larvae krill were observed closely associated with ice floes
M	Scotia Sea	Jun-Aug, 1988	Diatom and protozoan			Pack ice	Lancraft et al. (1991)	AMERIEZ program. Did not distinguish the food source
Mo, GF	WAP	Mar-Apr, Aug/Sept, 1984, 1985; Jan/Jul, 1987	GF: low ingestion rate suggesting carnivory do not occur as major winter dietary strategy.			Not specified	Quetin and Ross (1991)	Summary of 4 overwintering strategies.

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
SI	Weddell Sea	Mar, 1986	Smaller individuals (20 mm): higher δ^{15}N and δ^{13}C values; intermediate (40 mm): lower δ^{15}N and δ^{13}C values; large (> 50 mm): highest δ^{15}N and δ^{13}C values.			MIZ	Rau et al. (1991a)	AMERIEZ program. No SI baseline information, cannot assess the dietary sources.
M	WAP	Aug, 1992	Gut full of copepod remains.			Sea ice	Nordhausen et al. (1992)	Coastal water. Suggested only zooplankton could provide adequate food source for krill winter survival.
M	Scotia Sea	Nov, 1983	Diatom 39-50%, protozoan 32-48%			MIZ	Hopkins et al. (1993a)	AMERIEZ program

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M	Scotia Sea	Jun-Aug, 1988		Phytoplankton 31%, protozoan 30%, metazoan 24%, euphausiid debris 15%		MIZ	Hopkins et al. (1993b)	AMERIEZ program
I	WAP	Jul-Aug, 1992		Active feeding in winter. Carnivorous feeding on small copepod		Not specified	Huntley et al. (1994)	Coastal water. Adult krill are able to ingest small copepod *Oncaea* and *Oithona* spp.
M, I	Lazarev Sea	Apr, 1999	Contain crustacean fragments	Fuller guts, and contain more phytoplankton than adults		Pack ice	Atkinson et al. (2002)	Coastal water. Adult krill are able to ingest small copepod *Oncaea* and *Oithona* spp.

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M, FA, I	Lazarev Sea	Apr, 1999			FIII: diatom is the only recognisable food item. FA: dinoflagellate, copepod markers	Pack ice	Meyer et al. (2002)	Not able to identify the algae origin. Feeding experiment showed no significant preference on diet.
SI, I	Lazarev Sea	Apr, 1999	The SI data suggests all stages ingest pelagic POM as main food source. Furcilia larvae and juvenile had similar diet, adult was about half a trophic level higher.			Pack ice	Schmidt et al. (2003)	Turnover rate of SI was explored. Turnover rates are different in developmental stages, might be a problem for ecological interpretation.
FA	WAP	Apr, 1999; Apr-May, 2001	FAs ratios strongly depend on animal's total lipid content, and are of limited use as trophic index.			Not specified	Stübing and Hagen (2003)	This is a method paper.

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
FA, I	WAP	Apr, 1999; Apr-May, 2001	FA profiles do not reflect different feeding regimes.		FAs are influenced by their food	Not specified	Stübing et al. (2003)	
GF	WAP	Jul-Aug, 2001			Furcilia (IV to VI): low level of pigment content, suggesting larvae in the field were not feeding on sea-ice algae at maximum possible ingestion rates	Continental shelf with ice cover	Ross et al. (2004)	Assume the pigment content as an index of ice-algae feeding.

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
SI	Lazarev Sea	Apr-May, 1999	SI profile of male v.s. female			Not specified	Schmidt et al. (2004)	Multiple regions and seasons.
DNA	WAP	Nov-Dec, 2002		During peak bloom condition under the ice, diatom-affiliated sequences dominate diet, but heterotrophic taxa sequences were found as well.		Coastal water with ice cover	Martin et al. (2006)	This is a PCR-DGGE method paper.
I	lab		Feeding activity is low in winter light condition			No	Teschke et al. (2007)	The effects of simulated light regimes on feeding activities.

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
I	WAP	Apr-May, 2001			Furcilia has strong positive selection on small copepods and ciliates over algae.	No	(Wickham and Berninger, 2007)	
DNA	EA	Sep-Nov, 2007	Summer: mainly algae sequences; winter: large number of ciliates and krill sequences.			No	Vestheim and Jarman (2008)	DNA sequences of krill stomach contents were compared between summer and winter

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M	Lazarev Sea	Mar-May, 2004; Jun-Aug, 2006			CII FII (OW); CIII to FIII (ice): autotrophic flagellates and diatoms, protozoan, fragments of cnidarian, copepod, krill	Pack ice	Meyer et al. (2009)	Suggested under-ice topography is important for larval krill survival

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M, FA	EA	Sep-Oct, 2007	M: diatom fragments are major component; FA: diatom, flagellate, copepod, bacterial markers	M: diatom fragments are major component; FA: major: diatom markers, minor: flagellate and bacterial	M: diatom fragments are major component; FA: diatom, flagellate markers, bacterial markers	Pack ice	O'Brien et al. (2011)	No water samples were analysed. Assumed phytoplankton was ice-origin because of low biomass in the water column.
M, FA	Scotia Sea, Bransfield Strait	Mar, Jun-Aug, 2004; Apr, 2007; Jul-Aug, 2005, 2006; Nov, 2006	Benthic diatoms and particle found in krill stomach. FA: phytodetritus and bacteria markers.			Not specified	Schmidt et al. (2011)	All-year, including winter

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Table 1.1 – continued from previous page

Method	Region	Months	Krill stage			Sea ice zone	Ref	Note
			Adult	Juvenile	Larvae			
M, FA	Multiple regions	Mar, Apr, 2004; Jul, Dec, 2005; Jun-Aug, Nov, 2006	South Georgia (ice free): low lipid, contain phyto-plankton and seabed detritus; Lazarev Sea (ice cover all-year): high lipid, feeding activity reduced		FA: contain higher proportion of diatom and flagellate markers than post-larvae.	Different for regions	Schmidt et al. (2014)	Multiple regions and seasons. Regional dietary difference is confirmed

There is an increasing body of literature that recognises the important role of sea ice for larval krill winter survival (e.g. Quetin and Ross, 1991; Siegel and Loeb, 1995; Atkinson et al., 2004; Meyer et al., 2009). Studies on juvenile krill showed they feed through winter (Kawaguchi et al., 1986; Daly, 1990; Hopkins et al., 1993a; Stübing and Hagen, 2003), but their diet is debatable. Huntley et al. (1994) indicated juvenile krill actively feed on small copepods, where several studies showed they feed on a mixture of zooplankton, phytoplankton, and detritus (Kawaguchi et al., 1986; Daly, 1990; Hopkins et al., 1993a; Stübing and Hagen, 2003). In contrast to the conflicting views on adult krill overwintering strategies, larval krill are unable to store enough lipid and hence need to feed throughout the winter months (Meyer et al., 2002, 2009; O'Brien et al., 2011). Since food is scarce in the water column, sea-ice associated biota is considered critical for larval krill survival (reviewed in Meyer, 2012). The complex under-ice topography, in addition, provides a resting refuge for larval krill (Brierley and Thomas, 2002; Brierley et al., 2002; Quetin and Ross, 2009; Massom and Stammerjohn, 2010). Our current understanding of larval krill winter diet is derived from a small number of regional studies, limiting our ability to generalise the relationship between larval krill and sea-ice biota in the Southern Ocean (reviewed in Meyer, 2012). Consequently, many aspects of this relationship remain unclear. Outstanding questions include: 1) do larval krill rely on sea-ice biota as their food source in the winter? If not, 2) to what extent does sea-ice biota contribute to their winter diet? And 3) what is the composition of their winter diet? Answers to these questions could help predict the response of larval krill and in turn the krill population to changes in sea-ice conditions around Antarctica.

1.3 Zooplankton dietary studies

Baseline information on diet and trophic structure is fundamental for understanding food web relationships and the structure of ecosystems. Our current knowledge about the diet of zooplankton species heavily relies on dissection and visual analysis of gut contents and faecal pellets (Atkinson et al., 2012c; Pompanon et al., 2012). Other methods, including feeding incubation experiments, gut fluorescence, and morphology studies, are also widely used to investigate zooplankton feeding selectivity and ingestion rates of key species.

More recently, the use of trophic biochemical markers has been widely adopted in dietary studies. A 'perfect trophic marker' should satisfy the following criteria: 1) has a unique and easily identified origin; 2) does not harm organisms; 3) is not selectively processed during food uptake and incorporation; 4) is metabolically stable and transferable to higher trophic levels qualitatively and quantitatively (Dalsgaard et al., 2003).

Although no 'perfect' markers have yet been found, several less ideal markers, such as stable isotopes, signature fatty acids, and genetic markers have been used to study food web relationships in aquatic and terrestrial ecosystems. Both stable isotopes and fatty acids provide dietary information integrated over a period of several months or weeks. However, both methodologies suffer from low taxonomic resolution. Genetic markers, similar to the traditional gut and faecal content analysis, define what an animal has eaten just prior to being caught, and are also capable of identifying prey with high taxonomic resolution (Symondson, 2002; King et al., 2008; Pompanon et al., 2012)

1.3.1 Traditional methods

Gut content and faecal pellet analysis Gut content and faecal pellet analysis have been the most commonly used method, and have established the foundation of our knowledge of the diets of a variety of zooplankton species (e.g. Daly, 1990; Lancraft et al., 1991; Hopkins et al., 1993a,b). This approach uses light microscopy or scanning electron microscopy to examine the undigested items remaining in an animals stomach or faeces, which provides snap-shot information on the animals recently ingested diet. It is difficult to detect easily digested heterotrophic dietary components with this method (Price et al., 1988); therefore, the diets of many omnivorous/carnivorous zooplankton species remain largely unknown. For example, 70% of the analysed chaetognath specimens had unrecognisable contents in the gut in Kruse et al. (2010), thus providing limited taxonomic dietary information. A major bias this method suffers from is the post-capture feeding in the net codend (Omori and Ikeda, 1984; Hopkins, 1985; Hopkins and Torres, 1989). Zooplankton samples are concentrated into a small container during net sampling, providing opportunities for them to eat each other before analyses, which might not be a reflection of their natural diet. Due to the small size of zooplankton, and the wide taxonomic range of their prey composition, the gut and faecal analysis can be very labour intensive and time consuming. It also requires experience and highly developed taxonomic skills. This method, therefore, is of limited efficacy when conducting large-scale campaigns without skilled researchers. Additionally, the similarity in species composition of algae found in pack ice and the water column (Lizotte, 2001; Brierley and Thomas, 2002) makes it difficult to estimate the dietary importance of ice algae to zooplankton using this method.

Feeding and incubation experiment Feeding and incubation experiments are usually used to explore aspects of zooplankton feeding, such as, the ingestion rate and selective feeding of zooplankton species of interest (e.g. Meyer and El-Sayed, 1983). Using incubation experiments, Hamner et al. (1983) reported for the first time Antarctic krill

raking algae from the bottom of the ice, which established the later hypothesis that krill is able to utilise sea-ice algae as a food source for overwintering (Marschall, 1988; Stretch et al., 1988). Feeding experiments are normally conducted using certain types of prey in order to calculate assimilation rates and determine prey preferences. However, by their nature, incubation and feeding experiments use artificial environments, therefore, interpretation of an animals natural diets is biased to some extent (Atkinson, 1995). For example, results from feeding experiments suggested that Antarctic krill prefer to eat copepods (Price et al., 1988), but only limited copepod remains have ever been recorded in wild caught animals (Atkinson, 1995; Schmidt et al., 2006, 2014).

Gut fluorescence analysis Gut fluorescence analysis has been adopted to estimate the ingestion rates of herbivorous zooplankton (Mackas and Bohrer, 1976). This method uses a fluorometer to measure the Chlorophyll *a* (Chl *a*) equivalent phaeopigments to calculate the gut fullness or ingestion rate of the animal (Mackas and Bohrer, 1976; Conover et al., 1986). Since it has been developed for herbivorous animals, it provides information on ingestion rates of herbivorous items, but tends to overlook heterotrophic dietary components (Dubischar and Bathmann, 1997). In addition, this method is based on the assumption that all Chl *a* will degrade into phaeopigments, which are detectable by the method. It cannot delineate the origin or any taxonomic details of the dietary Chl *a*.

Morphology and behavioural observations Descriptions of morphological features and behavioural observations have helped us define the motile ability of zooplankton species and the size range of their diets (e.g. Hamner et al., 1983; Kils, 1983; Quetin and Ross, 1991; Hamner and Hamner, 2000). Morphology of many zooplankton species has been documented since early studies (Fraser, 1936; Marr, 1962). On the other hand, the complex behaviours of zooplankton species have never been fully understood despite being able to maintain them in laboratory aquaria. The detailed illustrations of Antarctic krills feeding basket by Kils (1983) revealed a variety of possible feeding techniques developed by the species. Kils (1983) suggested that krill could effectively filter items ranging between 1 μm and 30 μm in size, but they are also capable of grabbing bigger objects with the feeding basket, which makes cannibalism and carnivory possible. Morphological and behavioural observations probably cannot directly answer questions concerning specific dietary components, however, these observations are critical to our understanding of the structure of marine ecosystems.

1.3.2 Modern methods - trophic markers

Stable isotope analysis Stable isotope analysis has been found to be a useful tool for the study of trophic relationships in Antarctic food webs (Schmidt et al., 2003; Stowasser et al., 2012). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes are commonly used to provide insight into food web structure (Sydeman et al., 1997; Post, 2002b). In addition, the development of stable isotope mixing models (Phillips, 2001; Fry, 2013; Parnell et al., 2013; Semmens et al., 2013) has made it possible to quantitatively calculate the contribution of food sources based on stable isotope ratios. Carbon isotope ratios of animal tissues reflect closely those in their diet, and, therefore, can be used to trace carbon pathways and sources of primary productivity (Hobson and Welch, 1992; Post, 2002b). In particular, algae in polar sea ice and the underlying water column show distinct carbon signatures (Wada et al., 1987; Rau et al., 1991b; Kennedy et al., 2002; Pineault et al., 2013), which makes stable isotope analysis a plausible method to investigate zooplankton dietary contribution from sea-ice biota. Nitrogen isotope ratios show a stepwise enrichment between prey and consumer tissue and $\delta^{15}N$ is most frequently used as a trophic position indicator (Minagawa and Wada, 1984; Peterson and Fry, 1987). Stable isotope studies in the Southern Ocean have mostly concentrated on higher trophic levels, and only four studies have described zooplankton stable isotope signatures in detail (Wada et al., 1987; Rau et al., 1991b; Stowasser et al., 2012; Schmidt et al., 2003). Until now, no studies have investigated the trophic interactions in sea-ice associated biota using stable isotope analyses in the Southern Ocean. However, stable isotope ratios cannot provide detailed taxonomic information, which can only be accomplished to a certain degree by other methods, such as microscopy.

Lipid and fatty acid analysis During the last few decades, fatty acid signature lipid analysis (FAs) has been applied to study food web relationships in both freshwater and marine pelagic ecosystems (reviewed by Dalsgaard et al., 2003). The basic FA patterns in primary producers pass through food webs conservatively (Dalsgaard et al., 2003). This method cannot detail taxonomic dietary information at the species-specific level (Dalsgaard et al., 2003), but the presence and combinations of certain FAs can be characteristic of particular algal or zooplankton classes, and thus can track trophic interactions (e.g. Hagen et al., 2001; Schmidt et al., 2006; O'Brien et al., 2011). In the Arctic, ice algae is relatively richer in diatoms while open-water phytoplankton communities predominantly consist of dinoflagellates and small flagellates (Falk-Petersen et al., 1998; Henderson et al., 1998), therefore, differences in FA profile of diatoms and flagellates have been applied in studies on sea-ice trophic relationships (e.g. Graeve et al., 1994; Budge et al., 2008). In contrast, pelagic phytoplankton and sea-ice algae in

Antarctica have a more similar composition comprised of diatoms and flagellates, which make their FA profiles less distinguishable than in the north (Fahl and Kattner, 1993; Dalsgaard et al., 2003). However, FA analysis used alone is not sufficient to distinguish the origin of ice/water carbon sources in the Southern Ocean.

Molecular methods DNA sequences unique to a species can be used as a highly precise tool for species detection and identification. DNA-based techniques have been successfully applied to study predator-prey relationships in various ecosystems (reviewed by Symondson, 2002; King et al., 2008; Pompanon et al., 2012). These approaches are particularly valuable for small invertebrate predators in marine systems, where soft-bodied prey are hard to identify with traditional methods and direct in situ observation on feeding activity is usually impossible (Symondson, 2002; Gamboa et al., 2012; Connell et al., 2014).

DNA-based dietary analyses commonly rely on polymerase chain reaction (PCR) to amplify small regions of prey DNA from food remains in gut contents or faecal material of a predator (Pompanon et al., 2012). Species-specific primers are highly effective for detecting single prey species, but they are not an efficient way to study a wider range of prey (Harper et al., 2005; Deagle et al., 2009). Many zooplankton species are generalist predators consuming diverse diets, DNA sequences of which are very difficult to be fully reconstructed using species-specific primers (Symondson, 2002; Harper et al., 2005; Nejstgaard et al., 2007). To analyse a diet covering a wide range of taxa, it is more appropriate to use universal PCR primers or group-specific primers. Universal PCR primers are designed to amplify conserved DNA regions that cover as wide a range of species as possible (Deagle et al., 2014), and group-specific primers are designed to amplify a region of DNA from a higher taxon of interest including only a range of species (Jarman et al., 2004). Another challenge for DNA-based zooplankton dietary analyses is the difficulty of separating dietary components from the predator material due to zooplanktons small size. Broad coverage primers not only amplify desired DNA of prey, but also of the predator, which could dominate amplicons and prevent detection of prey DNA (Passmore et al., 2006; Vestheim and Jarman, 2008). PCR blocking primers are a useful technique designed to solve this problem. Predator-specific blocking primers can suppress PCR amplification of predator DNA molecules, and allow more inclusive primer sets to be used for amplification of prey DNA from dietary samples (Vestheim and Jarman, 2008).

As with the gut content analysis, DNA analyses can be biased by post-capture feeding. The major advantage of using DNA for prey identification is that these methods do not rely on morphological recognition. In addition, with the massive expansion of sequence

databases and the development of high-throughput sequencing technologies, it is now possible to obtain and accurately interpret massive amounts of sequence data in a cost effective manner and in a relatively short time (Deagle et al., 2009; Valentini et al., 2009b,a; Pompanon et al., 2012). The intent of many dietary studies is not to simply analyse diversity of the diet, but also to obtain quantitative data on the relative amounts of different prey types. With the high-throughput sequencing technologies, such as Next Generation Sequencing (Pompanon et al., 2012), and pyrosequencing (Deagle et al., 2009), it is possible to acquire the relative proportion of prey composition based on the proportions of the recovered DNA sequences. However, these quantitative sequence results still suffer from biological and technical bias, and require further investigation (Deagle et al., 2009, 2010, 2013; Jarman et al., 2013).

DNA-based methods alone cannot answer our particular question about zooplankton diet in the sea ice zone because of the similarity of species composition between the sea ice and the water column. However, DNA-based approaches could provide valuable complementary information if combined with stable isotope or FA analyses. DNA methods are able to resolve the poor taxonomic resolution from which both stable isotope and FA analyses suffer.

1.4 Ecosystem modelling

The physical habitats in the Southern Ocean ecosystem have been changing for the last 30 years, such as increases in surface ocean temperature (Bracegirdle et al., 2008), shifts in ocean currents (Böning et al., 2008; Sokolov and Rintoul, 2009), and regionally contrasting changes in sea-ice extent and seasonality (Stammerjohn et al., 2012; Massom et al., 2013; Turner et al., 2013). These changes have profound ecological implications for species at different trophic levels (Massom and Stammerjohn, 2010; Constable et al., 2014). In addition, fisheries on Antarctic krill, toothfish, and icefish have introduced, and will continuously add, pressures on Southern Ocean marine ecosystems. The krill fishery in particular possibly will become one of the largest fisheries in the Southern Ocean (Nicol and Endo, 1997; Nicol et al., 2011). In order to develop the best practical approach for fishery management and ecosystem conservation, we need to address the impacts of both environmental changes and human activities on the Southern Ocean ecosystem. Ecosystem models are useful tools for exploring potential food web and ecosystem responses to environmental changes and human impacts (Melbourne-Thomas et al., 2012; Murphy et al., 2012), and therefore, are widely used in many jurisdictions, such as the Commission for the Conservation of Antarctic Marine Living Resources

(CCAMLR; Constable, 2011), for the development and assessment of ecosystem-based management strategies.

For fishery and species management, age-structured population models have been popular tools. Population models focus on the species of interest, and divide the population into several age classes. Survival rates of each age class and transition rates between age classes can be derived from available data. The population dynamic, such as the growth rate of the entire population, could then be calculated using the population models (e.g. Caswell, 2001; Jenouvrier et al., 2003, 2009). However, this type of model focuses on and analyses a single species, and therefore, cannot assess the influence from other species within the system (Dambacher et al., 2009).

During the past few decades, the focus of scientific and political interest has shifted from the conservation of individual target species to entire ecosystems. With increases in computing power, there has been a rapid growth in ecosystem models for this purpose (Fulton et al., 2003; Dambacher et al., 2009). Ecosystem models are simplified representations based on food webs of either one part of an ecological community (e.g. Hill et al., 2007, 2012; Hill and Matthews, 2013; Melbourne-Thomas et al., 2015), or the entire ecosystem (e.g. Fulton, 2010; Rose et al., 2010; Ballerini et al., 2014). When more than one species is included in a model, more components and details of the ecosystem are included. Quantitative ecosystem models, therefore, require more information for estimates of parameters, which is generally difficult to obtain in practice (Fulton et al., 2003; Dambacher et al., 2009). Qualitative approaches, on the other hand, are not dependent on quantitative estimates of model parameters (Dambacher et al., 2009). Therefore, qualitative modelling can focus on describing general relationships of a system, and has become an attractive method for rapid model formulation and hypothesis testing regarding ecosystem structure and function (Melbourne-Thomas et al., 2013).

Our understanding of food web structures in the sea-ice ecosystem is limited. It is generally accepted that sea-ice algae serve as an important carbon source, and possibly the only carbon source for ice associated zooplankton species in the winter (Daly, 1990; Conover and Huntley, 1991; Quetin and Ross, 2009). Increasing evidence has recognised protozoas as a substantial dietary component of both krill and copepods (Atkinson et al., 2012c; Schmidt et al., 2014). However, our knowledge of microorganisms both in the water column and within the sea ice is limited (Garrison and Mathot, 1996; Thomas and Dieckmann, 2010). Winter field sampling in the Southern Ocean sea-ice zone, especially the pack ice zone, is rare, and will always be limited due to logistic difficulties. Therefore, using a modelling approach to identify the key processes and to direct future studies is essential. Qualitative modelling approaches, being able to formulate models and test

hypotheses without quantitative data, are attractive tools to help us understand sea-ice food webs.

1.5 Aims for this thesis

During last three decades, studies have shown a close relationship exist between zooplankton and the sea ice during winter/spring (e.g. Quetin and Ross, 2009; Flores et al., 2011, 2014; Swadling, 2014). However, the question of how zooplankton utilise sea-ice biota, and whether the sea ice is a major food source are still unclear. The overall objective of this thesis is to gain a better understanding of zooplankton feeding, Antarctic krill in particular, under the sea ice. In this thesis, I addressed this question with three key objectives:

(1) What are the morphological characteristics facilitating Antarctic krill feeding during the overwintering period? Chapter 2 examines the morphological changes through the development of Antarctic krill and relate the morphology to winter survival strategy.

(2) Do sea-ice biota contribute to the diet of Antarctic krill in all life stages? Chapter 3 investigates the trophic relationships between the under-ice zooplankton communities using stable isotope analysis.

(3) What feeding scenario favours Antarctic krill population over winter? Chapter 4 applies a qualitative modelling approach to examine the key processes influencing krill population dynamics under the sea ice during winter.

This thesis has been written as a series of separate manuscripts. Chapter 2 describes the morphology of krill through ontogenetic development from an egg to juvenile. Chapter 3 describes under-ice trophic relationships based on stable isotope analysis. Chapter 4 discusses the optimal feeding scenarios for krill population using qualitative modelling. The contribution of co-authors is outlined in the Statement of Co-authorship at the start of the thesis. A single bibliography is presented at the end of the thesis.

Chapter 2

A photographic documentation of the development of Antarctic krill (*Euphausia superba*) from egg to early juvenile

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2.1 Abstract

Antarctic krill (*Euphausia superba*) is a key species in Antarctic marine ecosystems, as well as an important species in the Southern Ocean fishery. Here, we provide the first detailed photographic documentation of embryonic and larval development of Antarctic krill over a 5-month developmental period under controlled laboratory conditions. Developing embryos and larvae were photographed every 3 h and every 5 days, respectively. Our results indicated a developmental time of approximately 6 days for embryos and 138 days for larvae (0.5 °C). This study provided baseline biometry information for future investigations of Antarctic krill development under changing environmental conditions.

2.2 Introduction

As one of the most abundant and important ecological and fishery species in the Southern Ocean, Antarctic krill (*Euphausia superba* Dana) has been studied since the Discovery Expeditions (1901 - 1904). Their life history was first fully recorded by Fraser (1936) based on net caught samples. This was followed by Marr (1962), who produced a more detailed description of the vertical distribution of the larval stages. Kirkwood (1982) summarised larval development based on these earlier studies and personal observations of euphausiid species. A comparison of larval development of the five most common euphausiids in the Southern Ocean was illustrated with short descriptions and is currently a commonly used reference for larval krill identification. Finally, George and Strömberg (1985) were the first and only document to illustrate and describe the embryonic developmental process of Antarctic krill.

Although recognised as a keystone species in Southern Ocean ecosystems, studies on the early life of Antarctic krill, especially the larval stages, still rely on illustrative references from last century (e.g. Frazer et al., 2002; Meyer et al., 2009). Until now, there has been no easy way to produce a detailed description of the embryonic and larval development of this species due to the technical difficulties of maintaining live krill in the laboratory, and obtaining fresh eggs in the field. Our study provides a detailed description of the morphology and growth of Antarctic krill under controlled laboratory conditions, and is the first to photographically record growth through its entire ontogenetic development.

Embryonic development represents the first step in the life cycle and is the period that is most sensitive to environmental perturbation in the life history of most aquatic crustaceans (García-Guerrero and Hendrickx, 2006). To understand how Antarctic krill cope with a changing environment, recent studies have focused on the impacts of warming temperatures and ocean acidification on the development of embryos (Kawaguchi et al.,

2011; Whiteley, 2011; Flores et al., 2012a; Kawaguchi et al., 2013). Notably, Kawaguchi et al. (2013) found that exposure to CO_2 level of 1,250 μatm and above causes deleterious impacts on embryonic development of Antarctic krill. However, it remains unclear how climate change might affect the developmental rate of an embryo, and how it might affect the different stages during embryonic development. A clear photographic record of embryonic development for this species will be valuable as a tool for stage recognition and comparison for future studies.

Antarctic krill larvae undergo a complex developmental process, which is made up of four phases and 12 specific stages (Fraser, 1936; Marr, 1962; Kirkwood, 1982). The newly hatched larvae go through two nauplius, one metanauplius, three calyptopis, and six furcilia stages. Two critical periods during this process affect larval survival and recruitment success: Calyptopis I, the first feeding stage, and furcilia stages, when they experience their first winter (Ross and Quetin, 1989, 1991; Daly, 2004). During both periods, they need sufficient food to maintain basal metabolism and support molting and further development (Ross and Quetin, 1989, 1991).

The purpose of this study was to photographically document the entire embryonic and larval development of Antarctic krill. Here, we also build on previous studies with updated detailed morphological and biometrical information throughout development. A series of high-resolution photographs are provided, accompanied by detailed descriptions of the developmental period from embryo through all larval stages of Antarctic krill incubated under laboratory conditions.

2.3 Materials and methods

Euphausia superba were collected with an 8 m^2 rectangular mid water trawl net (RMT - 8) in the Indian Sector of the Southern Ocean (64°09′S, 100°460′E), on April 7, 2011, from the RV *Aurora Australis*. After transportation back to Tasmania, female krill were incubated in the Australian Antarctic Division research aquarium. The facility was supported by two chilled sea water recirculating systems, and the seawater was monitored continually to maintain the temperature, salinity, and pH (King et al., 2003; Kawaguchi et al., 2010). Females were naturally fertilised in the aquarium in late December 2012. Every day throughout January and February 2012, gravid females were randomly selected with a hand net and carefully transferred into 1 L transparent plastic jars and housed individually. Jars were filled with filtered seawater and kept in the aquarium for 24 h. A 1 mm mesh screen was used to keep the female 10 mm above the bottom of the jar to protect spawned eggs from being eaten or damaged by the females. Jars were checked every hour for female spawning during this period. If no spawning occurred, all

females were released the next day and jars were refilled with new randomly selected females and filtered seawater. When spawning was observed, eggs were collected and females were released. Eggs were then observed under the microscope to determine whether fertilisation had occurred. All females, eggs, and larvae were incubated in the recirculated seawater system, at constant temperature, salinity, and pH (0.5 °C, 32, and 7.9, respectively).

The description of embryonic and larval development was based on a single brood of eggs (approximately 1,000), which was transferred to a 5 L container using a pipette within 2 h after spawning, and incubated in filtered seawater within the recirculated seawater system. Embryos were examined every 2 - 3 h for 7 days, and a subsample of 20 eggs was randomly chosen and photographed using a Leica M205C dissecting stereo-microscope with a Leica DFC 450 camera and Leica LAS V4.0 software. A modified petri dish was used for observation. The petri dish was built into a 100 × 100 mm hollow block with cold water running through the block to maintain a stable cold environment for samples. Eggs were observed and photographed under the microscope for approximately an hour and then discarded. The egg size was determined by measuring the diameter of the chorion using the software measuring function. In addition, another batch of eggs was also observed with a layer of fertilised jelly but did not show further cell cleavage. To test whether there was significant difference in mean egg diameter during development, a oneway ANOVA followed by a Tukey's Post Hoc test was applied.

After the nauplii had hatched, they were transferred into a 10 L tank with recirculated filtered seawater. Debris and dead animals were removed daily. Larvae were incubated without food until they reached the first calyptopis stage. After reaching this stage, the larvae were fed on a mixture of enriched *Artemia* and 500 mL cultured live algae (a mixture of *Phaeodactylum tricornutum*, *Geminigera cryophila*, and *Pyramimonas* sp. Approximately 30 mL L^{-1} of each alga) per day. *Artemia* was enriched by Instant Algae from Reed Mariculture Inc., USA (*Thalassiosira weissflogii*, *Pavlova* spp., and *Isochrysis* spp.) and FripPak fresh from Inve Aquaculture.

The development of the larvae was recorded via photomicroscopy. Every 5 days, a subsample of 10 - 20 larvae was randomly chosen from the tank with a pipette. For each animal, total length was measured, larval stages were inspected, and photographs were taken using the same Leica M205C microscope, camera, and attached software. During photographing, each larva was transferred onto the modified petri dish using a pipette. The petri dish contained drops of cold seawater to allow larvae to swim freely. Most of the photographs were taken when the larvae were naturally still. When larvae (mostly the late furcilia stages) showed strong swimming capability, a drop of 70% ethanol was added to the petri dish to calm them for optimal image quality. Larvae were kept in

the petri dish for approximately half an hour and then preserved in ethanol. Our larval staging study followed the description of *E. superba* in Kirkwood (1982).

2.4 Results

2.4.1 Embryology of Antarctic krill

Embryonic development of *E. superba* was completed in approximately 7 days. The eggs were spherical. Yolk was distributed evenly in the egg (centrolecithal), tightly surrounded by one vitelline membrane, one transparent membrane (chorion), and one layer of fertilsation jelly (Fig. 2.1a).

The nucleus was off-centre in the egg and darker in colour (Fig. 2.1a). Two polar bodies (20 μm) were associated with the vitelline membrane on the egg surface near the nucleus. The fertilised egg had a perivitelline space (PVS) that was 22 $\pm$ 4.17 μm in width (Fig. 2.1a). The calculation of PVS followed Gomez-Gutierrez et al. (2010). We also observed one batch of eggs with a layer of fertilised jelly but no evidence of cell cleavage. This batch of eggs had an extra thin layer between the embryo and vitelline membrane where a round particle penetrated. The size of these eggs was not significantly different from those that developed successfully (Student's t-test, $p < 0.01$). However, the unsuccessful eggs did have a significantly bigger PVS (26.3 $\pm$ 3.73 μm) compared to the successful ones (Student's t-test, $p = 0.006$).

The newly spawned eggs of *E. superba* were 615 $\pm$ 8.14 μm in diameter, with a 22 $\pm$ 4.17 μm PVS between the embryo and the egg membrane (Table 2.1). The size of an embryo did not change significantly until the gastrula stage (Tukey's Post Hoc test, $p < 0.01$) when PVS decreased constantly throughout the multiple-cell stages and then increased again when blastula and gastrula stages were reached. When the embryo reached limb-bud stage (Fig. 2.1m, n), the embryo increased by 8.8% in diameter and the PVS had nearly vanished.

Developmental sequence of krill embryo

Fertilisation Eggs were examined within 1 h after spawning, and the fertilisation jelly had already formed. This supported the observations in Tarling et al. (2009) that oocytes are fertilised during egg release.

Cleavage Cell cleavage occurred bilaterally and was holoblastic. The entire yolk divided equally at each cleavage, which occurred approximately every 2 - 3 h.

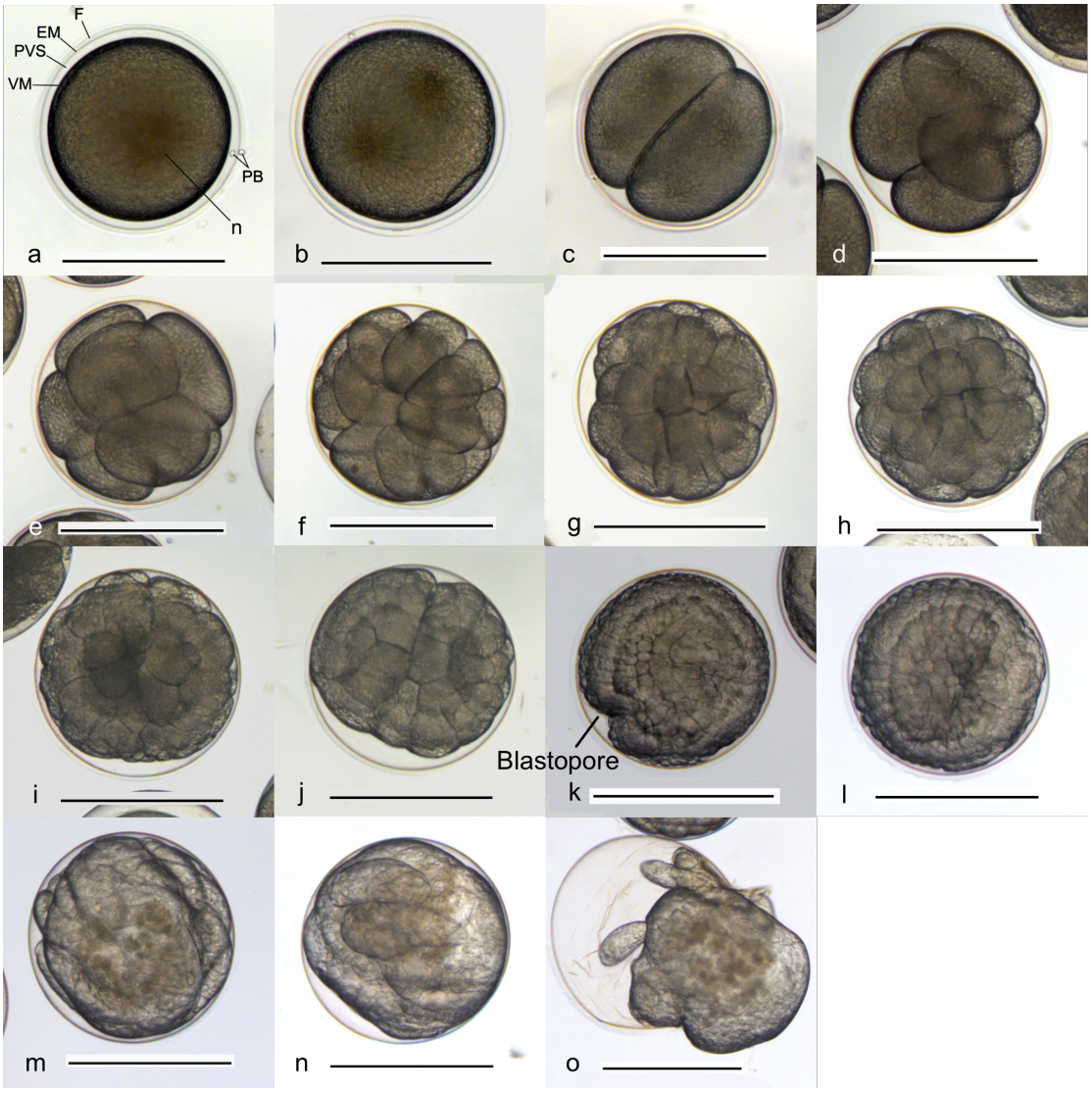

FIGURE 2.1: Embryological stages of the egg of krill *E. superba*. **a.** Newly spawned egg (*F* fertilisation jelly, *EM* embryo membrane, *PVS* perivitelline space, *VM* vitelline membrane, *PB* polar body, *n* nucleus), **b.** embryo starting cleavage, **c.** two-cell stage, **d.** four-cell stage, **e.** eight-cell stage, **f.** 16-cell stage, **g.** 32-cell stage, **h.** 64-cell stage, **i.** blastula, **j.** early gastrula, **k.** gastrula with a blastopore, **l.** late gastrula, **m.** and **n.** limb bud, **o.** hatching larva. Scale bar 500 μm.

Two–cell stage The first cleavage was meridian, which started with a small notch on the edge of the embryo within 2 - 4 h after spawning (Fig. 2.1b). Two nuclei separated and moved to opposite sides of the zygote (Fig. 2.1b). This first cell division was equal and resulted in two identical daughter cells. Cleavage was parallel to the animal-vegetal axis based on the position of polar bodies (Fig. 2.1c).

Four–cell stage Within the next 3 h, the second division occurred, which was perpendicular to the first cleavage plane and resulted in a four-cell embryo (Fig. 2.1d). This cleavage was slightly unequal and resulted in two small cells at the animal pole and two bigger cells at the vegetal pole. Nuclei were difficult to visualise.

TABLE 2.1: Developmental time, embryo, egg diameter and perivitelline space of the eggs of *Euphausia superba* at different stages of development (incubated under laboratory conditions, see "Materials and methods" section).

Stages	Accumulated time (h)	Number of eggs (μm) n	Embryo diameter (μm) Mean ± sd	Egg diameter (μm) Mean ± sd	Perivitelline space (PVS) (μm) Mean ± sd
1 cell		14	571 ± 5.1	615 ± 8.1	22 ± 4.2
2 cell	4	16	569 ± 6.6	602 ± 7.5	17 ± 4.5
4 cell	7	28	591 ± 7.2	616 ± 7.4	13 ± 3.0
8 cell	9	18	579 ± 8.5	604 ± 7.8	13 ± 3.1
16 cell	12	17	586 ± 7.9	609 ± 7.7	12 ± 2.4
32 cell	15.5	2	596	617	11
64 cell	19	10	591 ± 12	611 ± 5.9	10.5± 4.7
Blastula	22	18	585 ± 18	617 ± 12	17± 6.7
Gastrula	30	37	593 ± 14	625 ± 14	17± 5.3
Limb bud		10	621 ± 11	638 ± 13	14± 4.7
Died during hatching		8	598 ± 17	641 ± 26	27 ± 13
Fertilised but did not cleave		15	572 ± 10	625 ± 11	26 ± 3.7

Eight–cell stage The cleavage started from the animal pole and was slightly asynchronous with most of the embryos undergoing the third cleavage within 9 - 12 h of spawning. The third cleavage was perpendicular to both the first and second cleavage planes (Fig. 2.1e). Six-cell, seven-cell, and eight-cell embryos were observed at the same time.

16–cell stage Between 12 and 16 h following spawning, the fourth cell division occurred along two planes perpendicular to each other and resulted in a 16-cell embryo (Fig. 2.1f). This cleavage was not synchronous; therefore, multiple-celled embryos ranging from 10 to 16 cells were observed simultaneously.

32–cell stage This cleavage occurred within 15 h of spawning and resulted in a 32-cell embryo (Fig. 2.1g). It was difficult to detect the exact number of the cells after this stage due to the spherical distribution of the large number of blastomeres.

64–cell stage The embryo appeared like a "soccer ball" with similar-size blastomeres after 19 h following spawning (Fig. 2.1h).

Blastula A blastula stage (Fig. 2.1i) emerged after 22 h of spawning, featured with a hollow cavity (blastocoel) surrounded by an outer layer of cells. The blastula stage lasted approximately 20 - 21 h.

Gastrula Due to strong asynchronicity of cleavage, embryos at the 64-cell stage, blastula stage, and gastrula stage were observed at the same time. An early gastrula stage embryo was first observed at 30 h after spawning (Fig. 2.1j), when the inward movement started. The larger cells in the outside layer of the blastula began to migrate inward and folded into the vegetative pole, with the appearance of a blastopore (Fig. 2.1k). A late gastrula stage with multiple layers of small cells surrounding a cavity is shown in Fig. 2.1l. Gastrulation proceeded for approximately 2 more days before limb buds appeared.

Limb–bud stage Because of time gaps during observations, formation time of the limb-bud stage could not be defined exactly. On day 6 of incubation (approximately 121 h after spawning), the limb-bud stage had already formed and larvae appeared with three pairs of appendages folded beside or beneath the body (Fig. 2.1m, n).

Hatching Larvae hatched into the nauplius stage (Fig. 2.1o), with the earliest hatching at approximately 141 h following spawning. Time of embryonic development varied among individuals; however, the majority hatched at day 7. Larvae hatched out of the eggshell "backward" with the abdomen appearing first. Upon hatching, the nauplius swam backward with three pairs of appendages breaking out of the eggshell.

2.4.2 The larval development of Antarctic krill

At each developmental stage, the size of the larvae varied among individuals, and overlap of sizes was common between two stages (Table 2.2). However, the average size of larvae increased steadily during development from 0.742 mm at Nauplius I to 10.217 mm at Furcilia VI. The size increased substantially when larval krill moulted from Metanauplius (0.911 mm) to Calyptopis I (1.719 mm), and from Calyptopis III (2.708 mm) to Furcilia I (4.366 mm). A summary of key diagnostic features of each developmental stage is listed in Table 2.3.

TABLE 2.2: Developmental time and size (range and mean) of *Euphausia superba* at different larval stages (incubated under laboratory conditions, see "Materials and methods" section) (– indicates no developmental time recorded). Developmental time here is accumulated time from an embryo to the time when the stage was first observed.

Stage	Accumulated time (day)	Size range (mm)	Size mean (mm)
Nauplius I	0	0.669 - 0.797	0.742
Nauplius II	–	0.717 - 0.815	0.779
Metanauplius	9	0.830 - 0.978	0.911
Calyptopis I	13	1.556 - 1.936	1.719
Calyptopis II	35		
Calyptopis III	63	2.615 - 2.863	2.708
Furcilia I	85	3.578 - 5.255	4.366
Furcilia II	90	4.162 - 6.341	5.070
Furcilia III	97	5.667 - 7.918	6.900
Furcilia IV	117	6.305 - 8.495	7.616
Furcilia V	–	9.505	
Furcilia VI	138	9.809 - 11.001	10.217

Table 2.3: Summary of key diagnostic features of each larval developmental stage *E. superba*.

Phase	Stage	Key features		
		Body	Appendage	Spines
Nauplius	Nauplius I	Roundish	3 pairs	
		Unsegmented		
	Nauplius II	Oval	3 pairs	2 pairs of caudal spines
		Unsegmented		
Metanauplius	Metanauplius	Carapace	3 pairs	3 pairs of caudal spines
		Unsegmented		
				Telson
Calyptopis	Calyptopis I	Distinct cephalothorax	Mouthparts appeared	3 pairs of postero–lateral spines
		Unsegmented abdomen	Thoracic legs	6 inner terminal spines
		Eyes: two black spots		
	Calyptopis II	5 segments on abdomen		1 pair of lateral spines
		Compound eyes		3 pairs of postero–lateral spines
				7 terminal spines
	Calyptopis III	6 segments on abdomen		Uropods emerged
		Compound eyes within the carapace		1 pair of lateral spines
				3 pairs of postero–lateral spines
				7 terminal spines
Furcilia	Furcilia I	Mobile eyestalks	Pleopods (0–5 pairs)	1 pair of lateral spines

Continued on next page

Table 2.3 – continued from previous page

Phase	Stage	Key features		
		Body	Appendage	Telson
		Eyes extended out of the carapace		3 pairs of postero–lateral spines
				7 terminal spines
	Furcilia II	Fully developed eyes	Pleopods (2–5 pairs, at least 1 pair was setose)	1 pair of lateral spines
				3 pairs of postero–lateral spines
				7 terminal spines
	Furcilia III	Appearance similar to a juvenile	Pleopods (5 pairs, fully developed)	1 pair of lateral spines
		4 photophores		3 pairs of postero–lateral spines (the base of innermost postero–lateral spines is twice as wide)
				7 terminal spines (occasionally 6)
	Furcilia IV		Fully developed feeding basket	2 pairs of lateral spines
				2 pairs of postero–lateral spines (the base of innermost postero–lateral spines is three times as wide)
				5 terminal spines

Continued on next page

Table 2.3 – continued from previous page

Phase	Stage	Key features		
		Body	Appendage	Telson
	Furcilia V			2 pairs of lateral spines
				2 pairs of postero–lateral spines
				3 terminal spines
	Furcilia VI			2 pairs of lateral spines
				2 pairs of postero–lateral spines
				1 terminal spines
Juvenile	Juvenile			1 pair of postero–lateral spines
				1 terminal spines

Nauplius phase The nauplius phase included two stages. Throughout the nauplius phase, krill appeared with an unsegmented body and three pairs of appendages. The carapace was absent, and the cephalothorax was not distinct from the abdomen. Neither eyes nor mouthparts were observed at this stage.

Nauplius I Nauplius I had a roundish unsegmented body with three pairs of appendages (Fig. 2.2). No eyes, mouthparts, or spines were observed. The mean body length of Nauplius I was 742 μm (Table 2.2), similar to that of the embryo at its final stage. Krill remained at the Nauplius I stage for 3 - 5 days before transforming to Nauplius II.

FIGURE 2.2: Nauplius I *E. superba*. Scale bar 500 μm.

Nauplius II The unsegmented body of Nauplius II was oval and longer than Nauplius I. Nauplius II had three pairs of appendages (Fig. 2.3a). At the posterior end, a pair of long caudal spines appeared, accompanied by another pair of shorter spines outside and next to the long spines (Fig. 2.3b).

FIGURE 2.3: Nauplius II *E. superba*. **a.** *Dorsal view*, with three pairs of appendages and two pairs of caudal spines, **b.** two pairs of caudal spines at the posterior end. Scale bar 500 μm.

Metanauplius phase Metanauplius krill developed a carapace that surrounded the body (Fig. 2.4a). Protruding thoracic appendages were observed folded under the ventral side of the body (Fig. 2.4b). A third pair of small caudal spines appeared. Rudimentary eyes were observed from the ventral side under a scanning electron microscope.

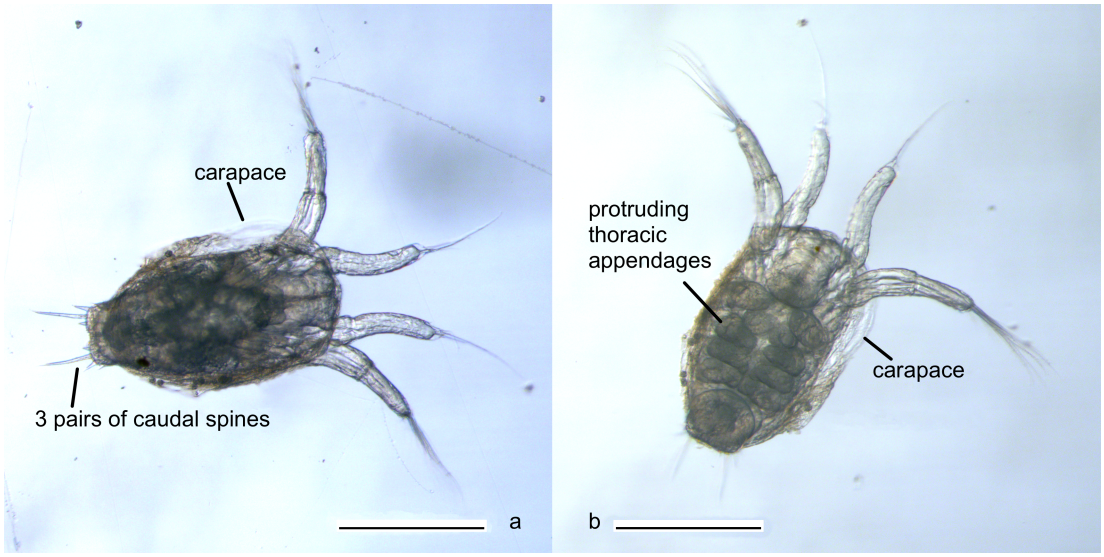

FIGURE 2.4: Metanauplius *E. superba*. **a.** *Dorsal view*, body surrounded by a carapace, three pairs of caudal spines at the posterior end **b.** *ventral view*, protruding thoracic appendages visible. Scale bar 500 μm.

Calyptopis phase The calyptopis phase included three stages. The larval krill began feeding during the first calyptopis phase. Calyptopis krill had a distinct cephalothorax

and abdomen. The eyes, mouthparts, abdomen, and uropods developed throughout the calyptopis phase. Abdominal appendages did not appear until the furcilia phase.

Calyptopis I The mean size of the krill increased to 1.719 mm as the abdomen unfolded in Calyptopis I (Fig. 2.5a). The cephalothorax was distinct from the unsegmented abdomen (Fig. 2.5a, b). Eyes appeared as two black spots, close to each other at the front of the cephalothorax underneath the carapace (Fig. 2.5a). Mouthparts and part of the thoracic legs were developed. Pigments first appeared on many specimens as little red dots, and the colour then faded to dark orange with the dots transforming into star-shape pigmented areas (Fig. 2.5c). However, the appearance of pigments was not uniform across the Calyptopis I stage and should not be considered diagnostic. Three pairs of long spines on the telson were observed on the telson, with six shorter inner terminal spines located in between them (Fig. 2.5c).

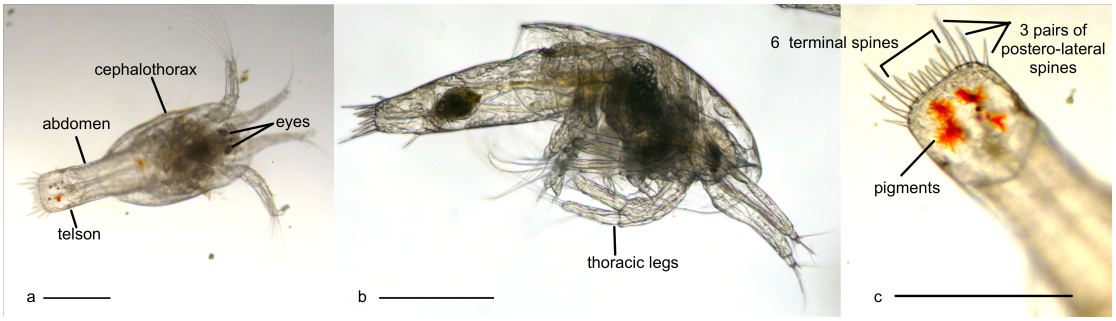

FIGURE 2.5: Calyptopis I *E. superba*. **a.** *Dorsal view*, with distinct cephalothorax and abdomen, eyes appear as black spots, **b.** *side view* with thoracic legs, **c.** telson with three pairs of postero–lateral spines, and six terminal spines. Scale bar 500 μm.

Calyptopis II By Calyptopis II, five individual segments had developed on the abdomen (Fig. 2.6a). The compound eyes developed further and were recognisable as distinct structures enclosed by the carapace (Fig. 2.6b). Two spines grew laterally from the middle edges of the telson, and one new terminal spine appeared at the end of telson, which increased the total number of terminal spines to seven (Fig. 2.6c).

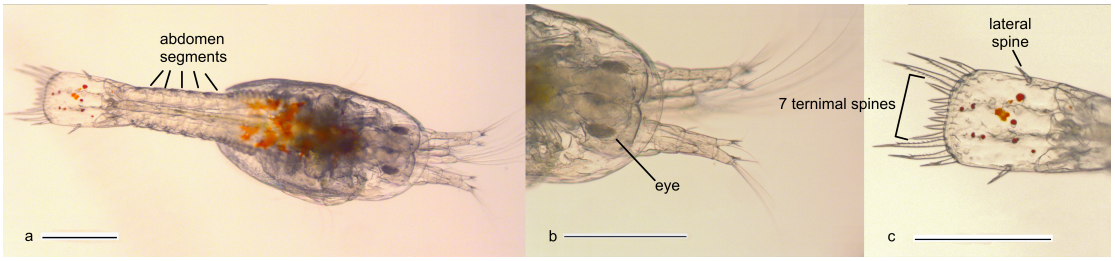

FIGURE 2.6: Calyptopis II *E. superba*. **a.** *Dorsal view*, abdomen with five segments, **b.** developed eyes, **c.** telson with one pair of lateral spines, three pairs of postero–lateral spines, and seven terminal spines. Scale bar 500 μm

Calyptopis III At the Calyptopis III stage (Fig. 2.7a), the sixth segment on the abdomen had developed, forming a distinct demarcation from the telson (Fig. 2.7b). The uropods emerged from the boundary of the telson and the sixth segment of the abdomen, and were parallel with the telson (Fig. 2.7c). Compound eyes were clearly observed under the carapace with red pigments appearing, but did not extend beyond the carapace (Fig. 2.7d).

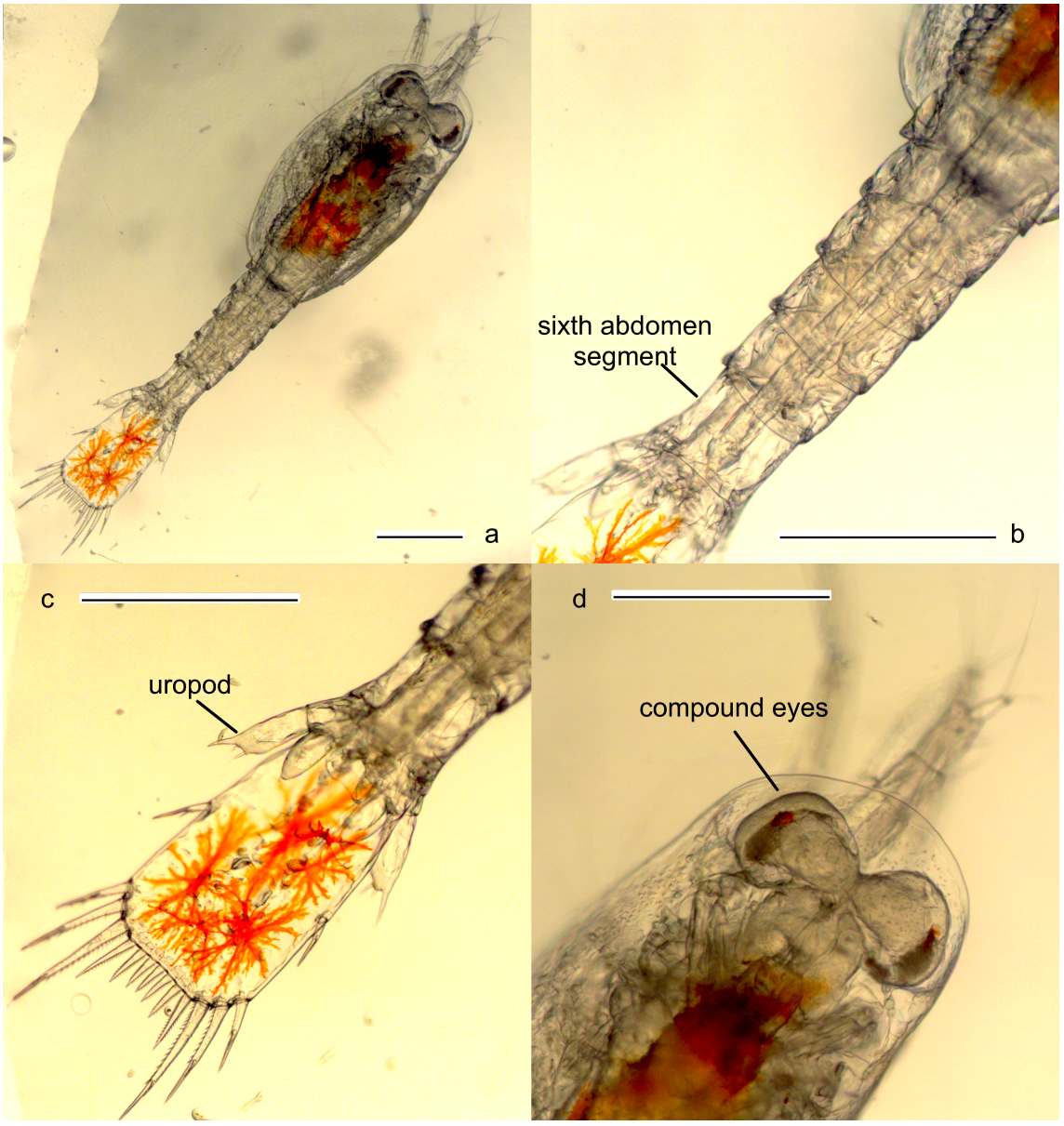

FIGURE 2.7: Calyptopis III *E. superba*. **a.** *Dorsal view*, **b.** abdomen with six segments, **c.** telson and uropods, **d.** developed compound eyes within the carapace. Scale bar 500 μm.

Furcilia phase Throughout the furcilia phase (six stages), the krill gradually took on the shape of a juvenile. They developed mouthparts, pleopods, and eyes in the first three stages of the furcilia phase. The setose process of pleopods from Furcilia I to II

differed among individuals. During the last three furcilia stages, the main distinguishable difference in morphology was a decrease in the number of terminal spines on the telson and the degeneration of the postero-lateral spines.

Furcilia I In Furcilia I, the last segment on the abdomen enlarged to nearly twice the length of the other segments (Fig. 2.8a). The eyestalks increased in size and became mobile, and "pinecone"-shaped eyes developed external to the carapace (Fig. 2.8b). At this stage, small pleopods protruded underneath the abdomen and the number of pairs of pleopods that were visible varied from 0 to 5; however, five pleopods were the most frequently observed (Fig. 2.8c; Table 2.4). Thoracic pleopods were also developed as lobes, and no setae were observed. The telson retained its seven terminal spines (Fig. 2.8d).

TABLE 2.4: Summary of pleopod combinations at Furcilia I and Furcilia II stage *E. superba*.

Larval Stage	Sample number	Number of pleopods	(%)
Furcilia I	35	5	37
		4	20
		3	8
		2	2
		1	2
		0	31
		Number of total pleopods, Number of setose pleopods, number of non-setose pleopods	
Furcilia II	51	5, 4, 1	27
		5, 3, 2	10
		5, 2, 3	6
		5, 1, 4	2
		4, 4, 0	16
		4, 3, 1	10
		4, 2, 2	8
		3, 3, 0	8
		3, 2, 1	2
		3, 1, 2	4
		2, 2, 0	8

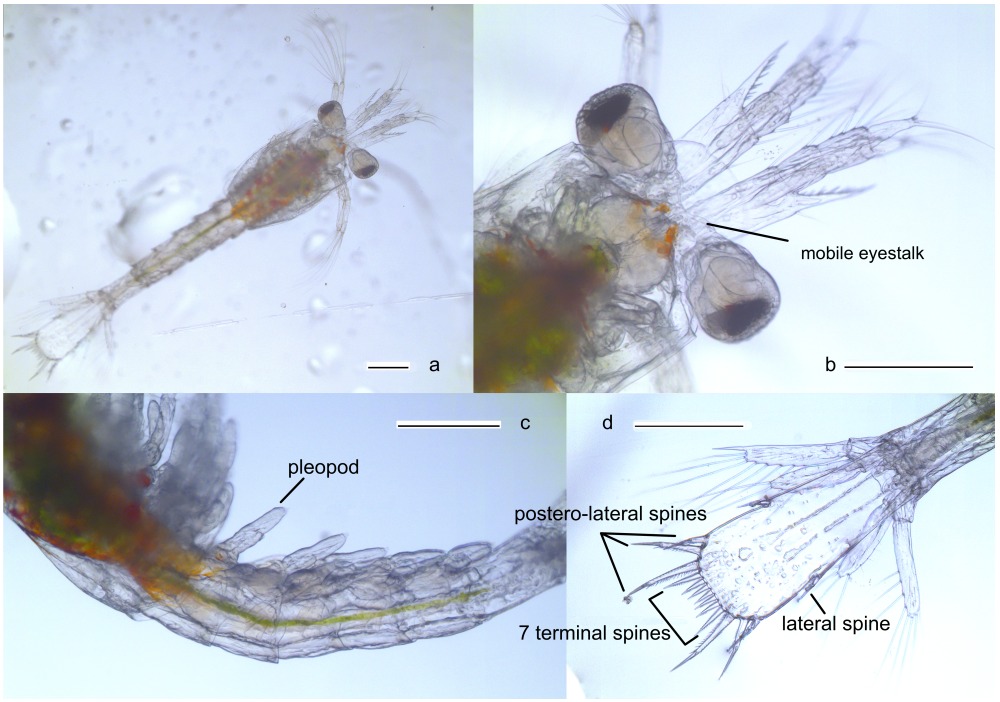

FIGURE 2.8: Furcilia I *E. superba*. **a.** *Dorsal view*, **b.** eyes and mobile eyestalks, **c.** protruded pleopods on the abdomen, **d.** telson with one pair of lateral spines, three pairs of postero–lateral spines, and seven terminal spines. Scale bar 500 μm.

Furcilia II By Furcilia II, at least one pair of pleopods was setose (number of pleopods ranged from 2 to 5) (Fig. 2.9a). Most of the krill observed had five pairs of pleopods, and the combination of setose and non-setose pleopods varied. Three pairs of setose pleopods with the last two pairs non-setose or four pairs setose with the fifth pair non-setose were commonly observed (Table 2.4). Thoracic appendages were further developed, but no setae were present. Photophores had developed on the abdomen (Fig. 2.9a). The eyes were fully developed, with red pigments clearly present (Fig. 2.9b). The telson still retained seven terminal spines, and the base of three pairs of postero-lateral spines was as wide as the other spines (Fig. 2.9c).

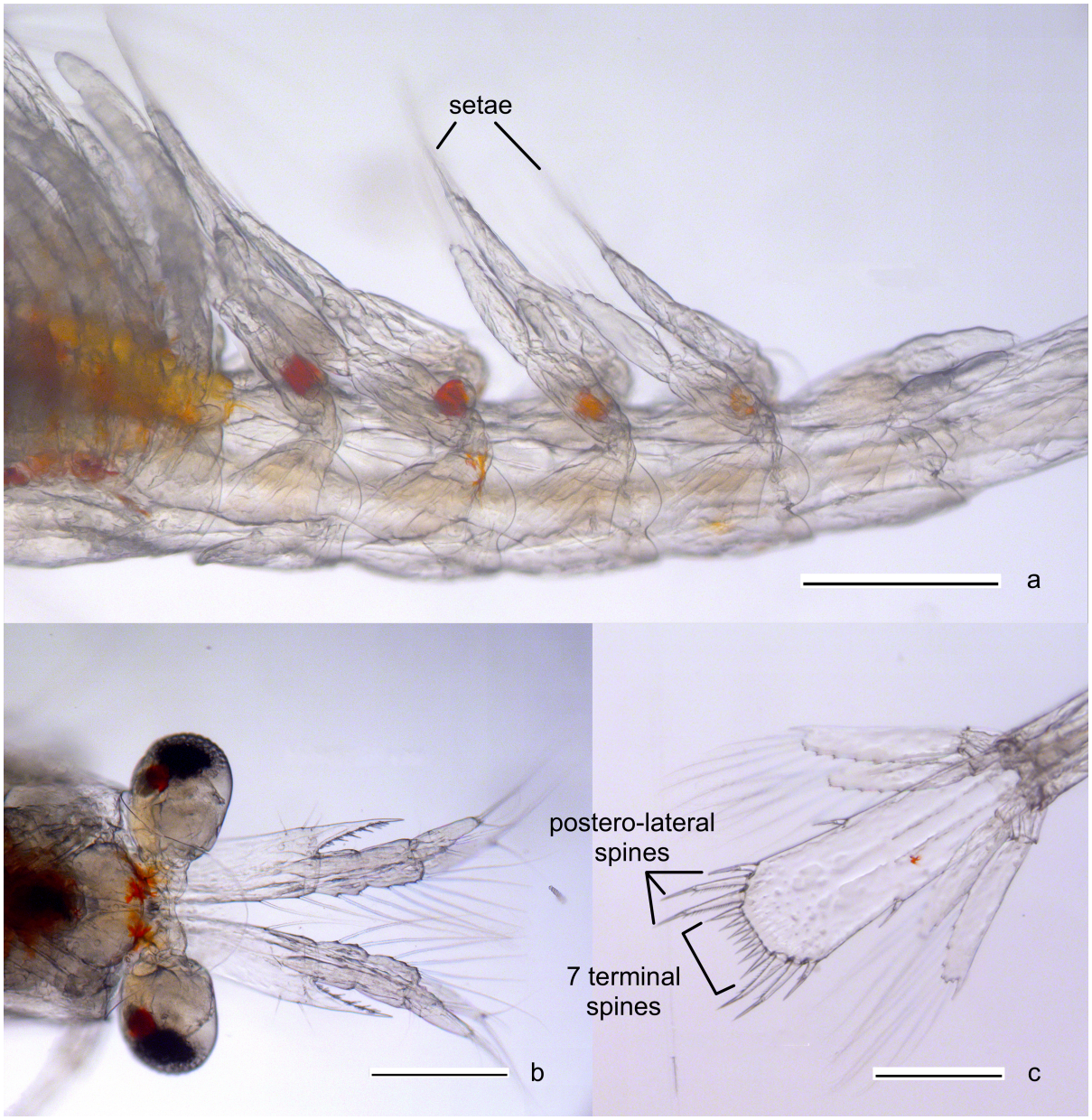

FIGURE 2.9: Furcilia II *E. superba*. **a.** Setose pleopods on the abdomen, **b.** fully developed eyes, **c.** telson with three pair of postero–lateral spines, and seven terminal spines. Scale bar 500 μm.

Furcilia III By the Furcilia III stage, the krill were similar in appearance to that of a juvenile (Fig. 2.10a). There were now five pairs of pleopods and four photophores on the abdomen. All pleopods had setae and were fully developed (Fig. 2.10a). Thoracic pleopods were further developed with setae forming the feeding basket. On the telson, the innermost postero-lateral spines were twice as wide at the base (Fig. 2.10b). There were seven terminal spines attached to the telson (Fig. 2.10b), though occasionally six terminal spines were observed (Fig. 2.10c).

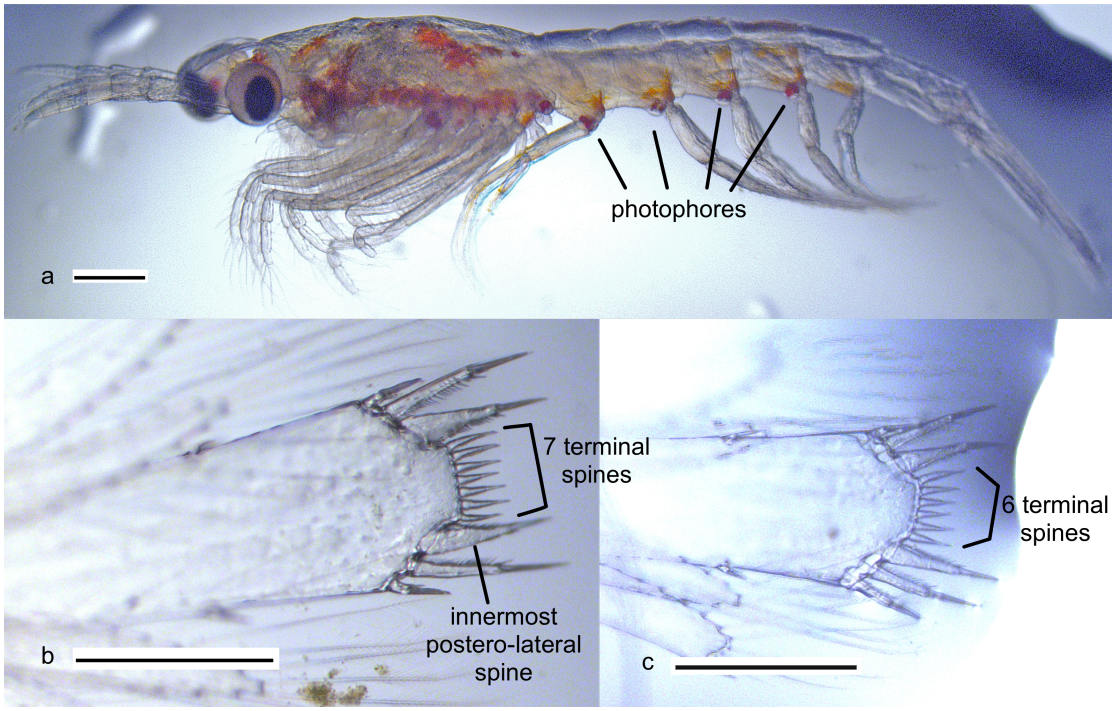

FIGURE 2.10: Furcilia III *E. superba*. **a.** Side view of Furcilia III, with four photophores and five developed pleopods, **b.** telson with three pair of postero–lateral spines, and seven terminal spines, the base of the innermost postero–lateral spines is twice wider, **c.** telson with three pair of postero–lateral spines, and six terminal spines. Scale bar 500 μm.

Furcilia IV Thoracic appendages were fully developed forming a functional feeding basket at the Furcilia IV stage (Fig. 2.11a). The tip of the frontal plate sharpened (Fig. 2.11b). There were five terminal spines remaining on the telson (Fig. 2.11c). The outermost postero-lateral spines degenerated and became lateral spines (Fig. 2.11c). The telson at this stage had two pairs of lateral spines with one in the middle and one pair at the rear. Two pairs of postero-lateral spines remained at the posterior of the telson, and the base of the innermost postero-lateral spines became three times wider than the others (Fig. 2.11c).

Furcilia V By this stage, the number of terminal spines had reduced to three, two pairs of postero-lateral spines and two pairs of lateral spines remained (Fig. 2.12a).

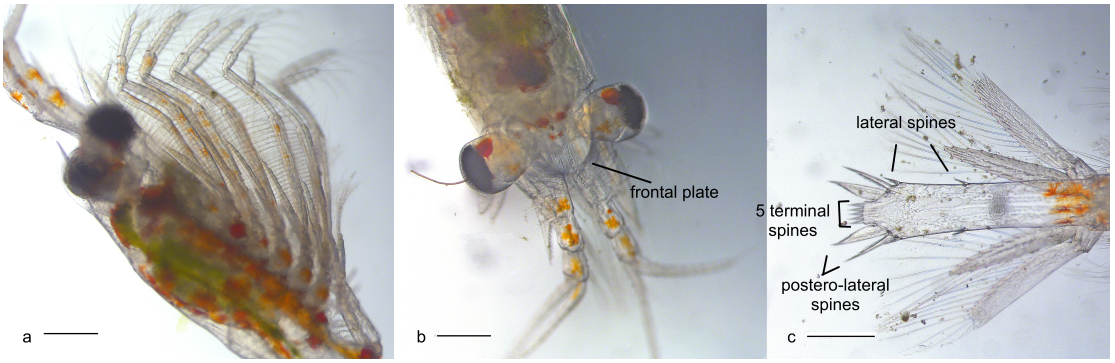

FIGURE 2.11: Furcilia IV *E. superba*. **a.** A fully developed feeding basket, **b.** sharpened frontal plate, **c.** telson with two pair of lateral spines, two pairs of postero–lateral spines, and five terminal spines. Scale bar 500 μm.

Furcilia VI This is the last stage of larval development of Antarctic krill. The telson had only one terminal spine, two pairs of postero-lateral spines and two pairs of lateral spines (Fig. 2.12b).

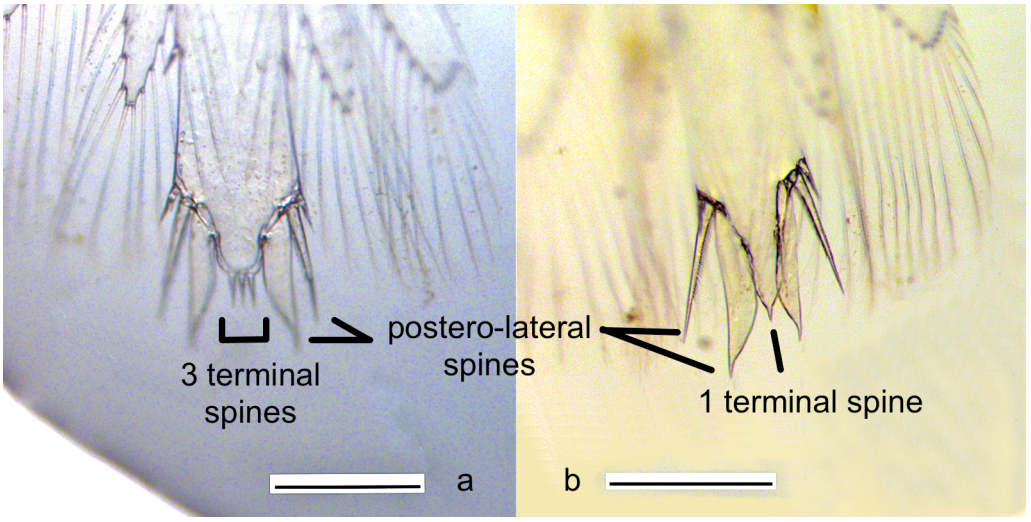

FIGURE 2.12: Furcilia V and Furcilia VI *E. superba*. **a.** Telson of Furcilia V, with three terminal spines and two pairs of postero–lateral spines, **b.** telson of Furcilia VI, with one terminal spine and two pairs of postero–lateral spines. Scale bar 500 μm.

Intermediate stages at furcilia phase

Variable forms of larvae were detected throughout early furcilia development. Furcilia I mostly had five pairs of pleopods; however, individuals with zero to four pairs of pleopods were observed. When larvae moulted into Furcilia II, setae appeared at the end of pleopods. There was variation in the total number of pleopods and the number of setose pleopods at this stage. Some Furcilia II had five pairs of setose pleopods, and most of Furcilia II developed setae on four pairs of pleopods with the fifth pair non-setose. Furcilia III retained seven terminal spines on the telson, although six terminal spines were observed occasionally.

Juvenile

The telson of juvenile krill had two pairs of lateral spines, only one pair of wide postero-lateral spines and one terminal spine. The thin postero-lateral spines had disappeared.

2.5 Discussion

2.5.1 Biometry of the embryos

The size of krill eggs is related to the female body size and nutritional condition of the female prior to spawning, which also varies annually and geographically (Mauchline, 1988). Our experiment was based on laboratory-spawned eggs from well-fed females. The embryos used in this study had a mean diameter of 615 ± 8.14 μm, which is consistent with sizes reported from field samples: 582 ± 5 μm in Marschall and Hirche (1984), 630 ± 15.63 μm in Quetin and Ross (1984), 556 - 803 μm from Harrington and Ikeda (1986).

The PVS is suggested to be an important embryological feature in euphausiids as it relates to the buoyancy of embryos (Harrington and Thomas, 1987; Nicol et al., 2004). As an example, the proportion of PVS differs dramatically among euphausiid species. Embryos of nearshore species have a bigger PVS for buoyancy, whereas embryos of pelagic species have a much smaller PVS so as they can sink quickly.

In addition, we suggest that the PVS changes during embryonic development and subsequently influences the sinking rate of an embryo. The sinking rate of *E. superba* embryos closely relates to the developmental stage, is fast during the initial cell cleavage, decreases at the gastrula stage, and increases again upon hatching (Ross and Quetin, 1982; Quetin and Ross, 1984; George and Strömberg, 1985). The PVS profiles determined in the present study (Table 2.1) are consistent with this trend. Despite their calculation of weight and density of the embryo, Quetin and Ross (1984) did not reach a satisfactory conclusion as to the mechanistic reason for krill embryos changing buoyancy. The change in PVS thickness agreed well with the pattern of sinking rates established by Quetin and Ross (1984). Therefore, we suggest the change in buoyancy through embryonic development is due to change in PVS volume.

Knowing the sinking rate is important for understanding the vertical distribution of krill embryos, and for predicting hatching depth (Marschall, 1983; Quetin and Ross, 1984). PVS is affected by thermal and salinity turbulence in the water (Marschall, 1983). The establishment of the relationship between PVS and sinking rate would improve our understanding of distribution of larval krill and the recruitment of this species. However,

due to the small sample size in this study, further investigation is needed to confirm this relationship between PVS and sinking rate.

2.5.2 Developmental time of embryos

The developmental time of krill embryos is a function of temperature and pressure (George and Strömberg, 1985; Ross et al., 1988; Yoshida et al., 2004), with high pressures and incubation temperatures accelerating development (Marschall and Hirche, 1984; George and Strömberg, 1985). In the present study, embryos raised in 0.5 °C seawater under 1 atm pressure completed embryonic development in approximately 6 days, which is indistinguishable from the predicted time of 5.93 days at 0 °C using the exponential model in Ross and Quetin (1988). Despite the lower temperature (0.5 °C), embryos in the present study cleaved at a faster rate (average 1 - 2 h faster at every stage) compared with observations made by George and Strömberg (1985). This may be due to differences in maternal provisioning. Further investigations focusing on the timing of embryonic development under different scenarios of temperatures and pressures would be useful to understand the developmental variations in the wild.

2.5.3 Developmental time of larvae

The length of the developmental stage of *E. superba* larvae is not isochronous (Ross et al., 1988). Calyptopis I was the longest stage (approximately 15 - 25 days) in the present study, which is consistent with results from past studies (Ikeda, 1984; Ross et al., 1988). It was reported that krill larvae could not successfully moult to Calyptopis II under periods of starvation or poor feeding conditions (Ikeda, 1984; Ross et al., 1988; Ross and Quetin, 1989). As the first feeding stage, Calyptopis I is considered to be a critical point for larval krill survival (Ross and Quetin, 1989). Yoshida et al. (2011) reported a 40% reduction of total lipid in Calyptopis I compared with lipid levels found in freshly spawned embryos. Ross and Quetin (1989) calculated carbon loss from the embryo to the Calyptopis I stage and reached a similar conclusion that larvae utilised about half of the initial lipid through this period of development. Therefore, Calyptopis I larvae possibly need a longer time to accumulate energy prior to transformation.

2.5.4 Intermediate stages

The second critical period during larval krill development is the furcilia stages, which occurs during the first autumn and winter, a time when the primary production in the water column is low and furciliae rely on the biomass in the sea ice for their survival

(Quetin et al., 1996; Daly, 2004; Lowe et al., 2012). There are six furcilia stages in krill larval development. The major questions that need to be addressed include whether there is any energetic difference between different furcilia stages, and if so, is there a particular furcilia stage that favours winter conditions in terms of their ability to optimise the food resource? Intermediate furcilia stages were detected in the present and past field studies (Fraser, 1936; Brinton et al., 1986; Daly, 2004; Meyer et al., 2009). The intermediate stages indicated that larvae followed different pathways through their development. The existence of intermediate stages was suggested as an adaptation to unfavourable living conditions, such as temperature, food quality, and quantity (Daly, 2004; Feinberg et al., 2006). Even though our experiment was conducted in the laboratory under favourable environmental conditions for krill larvae, the intermediate stages were still detected. Feinberg et al. (2006) hypothesised that different developmental pathways were due to inherent variability among individuals. In the present study, larvae were from a single brood and were raised under the same environmental conditions. Detection of variable intermediate stages during development in our study supports the possibility of inherent variability. The indirect developmental pathway was considered to be one of the strategies that larvae could use to time their development so that the appearance of the energy-demanding juvenile stages coincided with the onset of spring bloom. This could enhance their chances of coping with variable conditions and thus optimise survival (Brinton et al., 1986; Daly, 2004; Feinberg et al., 2006; Meyer et al., 2009).

Feinberg et al. (2006) suggested that Furcilia III is a developmental bottleneck for *Euphausia pacifica* as it is the longest furcilia stage and involves the most complex morphological changes, which agrees with our study of *E. superba*. However, further investigation on metabolism and energetic demand of furcilia larvae is needed to confirm whether the Furcilia III stage is also an energetically critical stage for *E. superba*. It is worth noting that laboratory-based studies of larval *E. superba* development have historically ended at the final calyptopis stages (McWhinnie and Denys, 1978; Ross and Quetin, 1982; Marschall and Hirche, 1984), while the development of furcilia larval stages has been generally overlooked. Several field investigations over last decade have focused on the physiology and energetic balance of furcilia larvae (Meyer et al., 2002; Daly, 2004; Meyer et al., 2009; Meyer, 2012). However, because larvae continue to grow and develop through the year, these studies have been more a reflection of seasonal differences rather than differences among stages. More laboratory-based experimental studies on furcilia stages would improve our understanding of metabolism, energy requirements, and larval overwintering strategies of this species.

Finally, our study is the first to combine photographic documentation and morphological descriptions of the embryonic and larval development of Antarctic krill. This work

provides an important baseline for comparison and interpretation of the morphology and development of Antarctic krill in a changing environment. Developmental rate of krill embryos is known to increase with temperature (Ross and Quetin, 1988). Additionally, krill embryonic development is known to be negatively impacted at elevated levels of CO_2, especially during the first 3 days of their embryonic development (Kawaguchi et al., 2013). However, how interaction between rising temperature and CO_2 concentration may affect the development of krill embryos is still unclear (Kawaguchi et al., 2013). In our study, larval krill underwent variable intermediate stages through the furcilia phase. This may be ascribed to inherent variability (Feinberg et al., 2006), rather than unfavourable living conditions as generally suggested (e.g. Daly, 2004). The survival of larvae depends on sea ice extent and phytoplankton blooms, which are highly variable seasonally (Ross and Quetin, 1991). The existence of intermediate stages may allow larvae to stay at an energy sufficient stage in order to optimise the available resources and hence maximise survival.

Chapter 3

The zooplankton food web under East Antarctic pack ice - a stable isotope study

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3.1 Abstract

Understanding how sea ice serves zooplankton species during the food-limited season is crucial information to evaluate the potential responses of pelagic food webs to the change of sea-ice conditions in the Southern Ocean. Stable isotope analyses ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were used to compare the dietary preferences and trophic relationships of major zooplankton species under pack ice during two winter-spring transitions (2007 and 2012). During sampling, furcilia of *Euphausia superba* demonstrated dietary plasticity between years, herbivory when feeding on sea-ice biota, and with a more heterotrophic diet when feeding from both the sea ice and the water column. Carbon isotope signatures suggested that the pteropod *Limacina helicina*, small copepods *Oithona* spp., ostracods and amphipods relied heavily on sea-ice biota. Post-larval *E. superba* and omnivorous krill *Thysanoessa macrura* consumed both water and ice biota, but further investigations are needed to estimate the contribution of each source. Large copepods and chaetognaths overwintered on a water column-based diet. Our study suggests that warm and permeable sea ice is more likely to provide food for zooplankton species under the ice than the colder ice.

3.2 Introduction

Knowledge of the trophic ecology of Antarctic zooplankton is essential for evaluating their functional roles in Southern Ocean food webs (Atkinson et al., 2012c), and for estimating their importance in energy flow and carbon flux through ecosystems (Turner, 1986; Dubischar and Bathmann, 2002; Buitenhuis et al., 2010). Antarctic zooplankton exhibit profound seasonal and regional variability in their diets (Hopkins et al., 1993a,b). During food-limited seasons zooplankton adopt a variety of survival strategies. Only herbivores, such as *Calanoides acutus*, undergo winter diapause and mainly rely on large lipid reserves to survive (Schnack-Schiel and Hagen, 1995; Pasternak and Schnack-Schiel, 2001). Other species appear to be able to either extend their feeding cycle into the winter months (e.g. *Calanus propinquus*), or overwinter within or under the sea ice (Atkinson, 1998; Swadling, 2014). Antarctic pack ice provides zooplankton with an important refuge from predators and currents (Brierley et al., 2002; Meyer et al., 2009; Swadling, 2014). In addition, the high algal biomass contained within the sea ice provides a possible alternative diet for zooplankton in the underlying water during phytoplankton-deplete seasons (Thomas and Dieckmann, 2010; Meiners and Vancoppenolle, 2012). Many crustaceans, especially in their early life stages, are closely associated with the sea ice (Schnack-Schiel et al., 2001, 2008; Flores et al., 2011, 2014).

Antarctic krill (*Euphausia superba*), for instance, directly feed on ice algae during winter (Daly, 1990; Meyer et al., 2009). However, it remains unclear whether sea ice is a major feeding ground for zooplankton during the winter, and to what extent zooplankton interact with sea ice (Brierley and Thomas, 2002; Atkinson et al., 2012c).

Most of our understanding about zooplankton diets is based on traditional approaches, such as analyses of gut contents and faecal pellets (e.g. Hopkins et al., 1993a; Pasternak and Schnack-Schiel, 2001), bottle feeding experiments (e.g. Lonsdale et al., 2000; Meyer et al., 2003), and gut fluorescence measurements (e.g. Pakhomov et al., 1997; Lee et al., 2013). Gut contents and faecal pellets analyses provide snap-shot information on recently ingested food, but underestimate easily digestible food items or long-term diets (Omori and Ikeda, 1984). Feeding experiments enable the calculation of feeding rates and food selectivity, but may not be a reflection of ingestion of naturally available food sources. Gut fluorescence analysis quantifies the herbivorous components, but tends to overlook the heterotrophic elements in zooplankton diets. In addition to these limitations, the similarity in species composition of algae found in pack ice and the water column (Lizotte, 2001; Brierley and Thomas, 2002) makes it difficult to estimate the dietary contribution of sea-ice biota to zooplankton using traditional methods.

More recently, stable isotope analysis has been found to be a useful tool for the study of dietary relationships in Antarctic food webs (Schmidt et al., 2003; Stowasser et al., 2012). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes are commonly used to provide insight into food-web structure (Sydeman et al., 1997; Post, 2002b). Carbon isotope ratios of animal tissues reflect closely those in their diet and experience minor trophic enrichment of 0 to 1‰ per trophic level, and, therefore, can be useful for tracing carbon pathways and sources of primary productivity (Hobson and Welch, 1992; Post, 2002b). In particular, algae in the sea ice and the water column show distinct carbon signatures (Wada et al., 1987; Fischer, 1991; Rau et al., 1991b; Kennedy et al., 2002; Pineault et al., 2013). Sea-ice core samples obtained from the Southern Ocean generally show a more positive $^{13}C/^{12}C$ values (-28 to -16‰, Fischer, 1991; Rau et al., 1991b; Kennedy et al., 2002) than phytoplankton in the surface water, which is characterised by strong negative $^{13}C/^{12}C$ ratios (-30 to -21‰, Wada et al., 1987; Fischer, 1991; Rau et al., 1991b). Although stable isotope signatures cannot reveal dietary information at high taxonomic resolution, the separation in isotopic signals between sea ice and water samples makes stable isotope analysis a plausible method to investigate zooplankton dietary contribution from sea-ice biota. Nitrogen isotope ratios show a stepwise enrichment between prey and consumer tissues and $\delta^{15}N$ is most frequently used as a trophic position indicator (Minagawa and Wada, 1984; Peterson and Fry, 1987). Stable isotope studies in

the Southern Ocean have mostly concentrated on higher trophic animals, and only four studies have described stable isotope signatures of pelagic food webs in detail (Wada et al., 1987; Rau et al., 1991b; Schmidt et al., 2003; Stowasser et al., 2012). Using carbon and nitrogen stable isotope analyses, the present study provides the first investigation of the trophic ecology of several major zooplankton species in the East Antarctic pack ice zone during the winter-spring transition. The aims of this study are 1) to investigate the contribution of sea-ice biota to krill and other zooplankton under Antarctic pack ice, and 2) to assess the trophic relationships within the under ice zooplankton community.

3.3 Materials and methods

Samples were collected during two East Antarctic sea ice voyages on board the RV *Aurora Australis*. The Sea Ice Physics and Ecosystems eXperiment (SIPEX) was conducted from 4 September to 17 October, 2007, and SIPEX-2 from 14 September to 16 November, 2012. The research was conducted off East Antarctica between 115° and 130°E, 64° and 66°S, with 14 ice stations and 6 ice stations sampled during SIPEX and SIPEX-2, respectively (Fig. 3.1).

Sampling during SIPEX At each station, a 10 L Niskin bottle was deployed through ice holes and used to collect surface seawater samples from 5 m depth below the sea surface for measurements of particulate organic matter (POM). This depth was chosen to be distinct from the overlaying sea ice, which could reach up to 4 m in areas of ridging. A known volume of seawater was pre-filtered through a 50 μm mesh to remove zooplankton, then filtered onto pre-combusted (450 °C for 12 h) glass fibre filters (Whatman GF/F, nominal pore size 0.7 μm). Three sea-ice cores were collected at each ice station using a Kovacs Mark II corer (0.09 m internal diameter). The lower 0.15 m of each core was cut with a stainless steel saw, placed in clean plastic containers, and melted in pre-filtered seawater (0.2 μm) at 4 °C in the dark. Subsamples of known volume were subsequently filtered onto pre-combusted (450 °C for 12 h) glass fibre filters. Filters were rolled into cryotubes and stored in liquid nitrogen until processed in the home laboratory.

At each station, an umbrella net (100 μm mesh) was deployed below the ice from the ice surface to a depth of 50 m to collect mesozooplankton. A Surface and Under-Ice Trawl (SUIT, van Franeker et al., 2009, 300 μm mesh) and a Rectangular Midwater Trawl (RMT- 8+1, Baker et al., 1973, RMT 1 = 330 μm mesh) were used to collect krill species (*E. superba* and *Thysanoessa macrura*) from below the ice and the upper 200 m of the water column (for details see O'Brien et al., 2011). Some larval *E. superba* were collected opportunistically during ice-core collection. Catches were then sorted alive

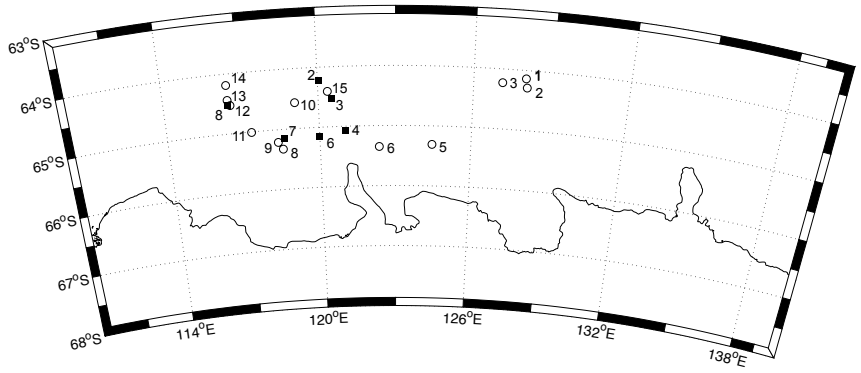

FIGURE 3.1: Map of the study areas in the Southern Ocean pack ice zone. Dots show positions of sampling sites during SIPEX (open circle) and SIPEX-2 (filled square). Numbers indicate the sampling site numbers.

under a dissecting microscope. Zooplankton were identified to species level whenever possible (following World Register of Marine Species). Developmental stages and sex of *E. superba* were recorded. Several individuals of zooplankton taxon were then pooled in cryotubes and stored in liquid nitrogen until analysis.

Sampling during SIPEX-2 Seawater was sampled from the ship-based scientific seawater line, of which the water intake was located 5 m beneath the sea surface. At each station, a known volume of seawater was pre-filtered through a 50 μm mesh to remove zooplankton, and then filtered onto pre-combusted (450 °C for 12 h) quartz microfibre filters (with a nominal retention size of 0.3 μm). Filters were stored in cryotubes in liquid nitrogen until processing. Sea-ice cores were collected and stored using the same method as during SIPEX, only all the samples were filtered onto pre-combusted (450 °C for 12 h) quartz microfibre filters. Although different filters were used for POM collection between years, and there was a small difference in retention size of the filters, it has been shown that δ^{13}C values of POM are not significantly affected by filters of different material or this degree of difference in pore size (Altabet, 1990).

Zooplankton were collected using several techniques, including deploying an umbrella net (100 μm) and a ring net (100 μm) from the ice surface to 60 m depth, using a modified commercial fish pump (Aqua-life BioStream), and direct trapping via a hand net through ice holes. Additionally, *E. superba* juveniles and larvae (furcilia VI) and *T. macrura* juveniles were collected directly from under the ice when ice cores were retrieved. Zooplankton were then sorted live under a dissecting microscope. Samples were identified to the lowest taxonomic level possible, and individuals of each zooplankton taxon were then pooled and stored in cryotubes. Developmental stages and sex of *E. superba* were recorded. For *E. superba* and *T. macrura*, the stomach and digestive gland of each specimen were removed for other dietary analyses. The remaining part of the body was individually stored in cryotubes and kept in liquid nitrogen until analysis. Little food remains were detected using microscopic and genetic analysis of the removed stomachs and digestive glands (data not shown); thus we conclude that removal of organs had negligible impact on stable isotope ratios of sampled euphausiid species.

Stable isotope preparation POM filters were demineralised in the laboratory in HCL (1%) fumes for 24 h, and then dried at 60 °C for at least 24 h. Subsamples were taken from each filter and encapsulated in silver cups (Kennedy et al., 2005).

Zooplankton samples were thawed and rinsed with Milli-Q water, then refrozen in liquid nitrogen. All zooplankton samples were then freeze-dried (JAVAC SB9) to constant mass at -40 °C for 24 h. Each zooplankton sample was weighed and transferred into a

pre-weighed tin cup. Specimens beyond the required weight were homogenised to fine powder and a subsample was taken from the powder. Small individuals of the same species were pooled together to obtain sufficient material for analysis (0.5 to 1 mg dry sample weight). Zooplankton samples were not acidified because acidification treatments are still under debate and their effect on both carbon and nitrogen isotopic compositions are not clear (Mintenbeck et al., 2008; Brodie et al., 2011). Minor carbonate content in zooplankton species generally has minimum impact on their δ^{13}C values, but the shelled pteropod *Limacina helicina* is an exception (Pomerleau et al., 2014). Therefore, carbonate corrected δ^{13}C values were calculated for *L. helicina* using the equation in Pomerleau et al. (2014):

$$\delta^{13}C_{carbonate-corrected} = 0.994 \times \delta^{13}C_{raw} - 1.096 \tag{3.1}$$

Lipid was not extracted prior to stable isotope analysis because, firstly, it is unclear how nitrogen isotopic compositions respond to the lipid-extraction treatment (Sweeting et al., 2006; Post et al., 2007; Mintenbeck et al., 2008); secondly, the low C:N ratios of our samples suggested that the majority of our zooplankton species had low lipid content and extraction was expected to have limited influence on δ^{13}C values (Post et al., 2007; Logan et al., 2008). However, we used several mathematical models (Post et al., 2007; Logan et al., 2008) to correct our δ^{13}C values and present these lipid-corrected δ^{13}C values in the Results (presented in section 2.5).

Stable isotope analysis Carbon and nitrogen stable isotopes were analysed using an Iso-Prime100 mass-spectrometer coupled with an elemental analyser (Elementar vario PYRO cube, Germany) at the Central Science Laboratory, University of Tasmania (Australia). Samples were flash combusted and converted into N_2 and CO_2. Gases were introduced into the elemental analyser to obtain the quantitative data on element content in the samples (which was used to calculate sample C:N ratios), and sequentially were released into the mass spectrometer to perform the isotopic measurements. Isotope ratios were reported as parts per thousand (‰) deviations from the conventional standards, Pee Dee Belemnite (PDB) for carbon and atmospheric nitrogen gas for nitrogen. Stable isotope concentrations were expressed in delta (δ) notation as parts per thousand according to the following equation:

$$\delta X = (\frac{R_{sample}}{R_{standard}} - 1) \times 1,000 \tag{3.2}$$

where X=^{13}C or ^{15}N, and R= ^{13}C/^{12}C or ^{15}N/^{14}N. Internal laboratory standard with known isotopic composition were measured for instrument calibration after every 5^{th} sample. The stability of the instrumentation, analytical precision, drift correction and linearity performance were calculated from the repetitive analysis of these standards.

For samples collected during SIPEX-2, we could only retrieve δ^{15}N values from four sea-ice POM samples because the amount of nitrogen retained on the filters was generally below the quantification limit of the instrument. For the same reason, no δ^{15}N values could be obtained from water POM samples.

Lipid correction All lipid-correction models used C:N as a proxy to calculate the lipid content in a sample and predict lipid-corrected δ^{13}C. Model 1 (Post et al., 2007) was a lipid-correction linear equation developed for aquatic animals:

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C - 3.32 + 0.99 \times C:N \tag{3.3}$$

Model 2 was a lipid-correction equation for aquatic invertebrates described in Logan et al. (2008):

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 3.388 - \frac{3.388 \times 3.314}{C:N} \tag{3.4}$$

Additionally, Logan et al. (2008) suggested two krill-specific models that perform more accurately to calculate δ^{13}C $_{lipid-corrected}$ for euphausiid species:

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 3.713 \times (-0.051 + \frac{3.90}{1 + \frac{287}{L}}) \tag{3.5}$$

where $L = 93_{\frac{}{1+(0.246\times C:N-0.775)^{-1}}}$

Here, L represents sample lipid content.

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 6.941 - \frac{6.941 \times 3.346}{C:N} \tag{3.6}$$

Supporting measurements Air temperature, surface water temperature, and water salinity were measured by the ship-based underway data system (Reeve, 2009, 2013, available through the Australian Antarctic Data Centre). Ice and snow thickness, ice salinity and brine volume fraction are reported in Meiners et al. (2011) for SIPEX, and Ugalde et al. (this issue) for SIPEX-2. Ice-core Chlorophyll *a* (Chl *a*) concentration

was reported in Meiners et al. (2011) for SIPEX, and measured with the same method for SIPEX-2 (Appendix A). We calculated Chl *a* concentration in the bottom 0.10 m of ice cores, integrated Chl *a* over the entire ice thickness, and calculated the percentage of integrated Chl *a* in bottom 0.10 m ice from data reported in Meiners et al. (2011) and Appendix A. Under-ice water Chl *a* at 10 m depth was reported in Meiners et al. (2011) for SIPEX, and measured from CTD-rosette samples for SIPEX-2 (Johnson, unpublished data). All data are provided in Appendix A.

Statistical analysis Kruskal-Wallis tests were used to test for significant differences in environmental variables among sites and between years. ANOVA, followed by Tukey's Post Hoc test, was used to compare the means of δ^{13}C and δ^{15}N values of both water and ice POM among sites and between years (Zuur et al., 2007). Means of the stable isotope ratios among zooplankton species were compared within each year using Student-Newman-Keuls tests following Schmidt et al. (2003). Statistical analyses were conducted using R (version 3.0.2).

To estimate the contribution of each food source to the diet of zooplankton, a Bayesian stable isotope mixing model (SIAR package in R, Parnell et al., 2010, 2013) was applied to our isotopic data. The SIAR model involved the input of δ^{13}C and δ^{15}N values of potential dietary sources and trophic fractionation factors to generate probability distribution for each potential food source in the consumer diet. Here, pelagic and sea-ice POM were considered as potential food sources. Because the uncertainty of trophic fractionation factors in the Southern Ocean, we tested three sets of values for δ^{13}C and δ^{15}N, 3.4 ± 1‰ and 1.5‰ (McConnaughey and McRoy, 1979), 2.2 ± 0.3‰ and 0.5 ± 0.13‰ (McCutchan et al., 2003), and 3.4 ± 1‰ and 0.4 ± 1.3‰ (Post, 2002a), respectively. The contributions of different food sources were reported as means with 95% confidence interval.

3.4 Results

3.4.1 Environment and ice condition

Air temperature varied throughout the sampling periods in both years, with an approximately 10 °C increase during each voyage (Appendix A). This temperature increase over time indicated the winter-spring transition (Worby et al., 2011). Water temperatures and surface salinities remained stable at around -1.8 °C, and 34.2 respectively, and did

not vary between years.

Although water column conditions were similar, physical conditions of the sea ice were different between years. During 2007, average ice thickness was 0.86 m (range: 0.39 to 1.99 m), with an average snow cover of 0.04 m (Meiners et al., 2011). In 2012, average ice thickness was 1.38 m (range: 0.75 to 2.18 m), with an average snow cover of 0.46 m (Ugalde et al., this issue). Sea ice in 2012 was generally warmer with higher brine volumes compared to 2007; however, ice bulk salinity did not differ between years (Appendix A).

Integrated Chl *a* (integrated over the entire ice thickness) was similar between years (mean ± SD: 2.86 ± 3.93 mg m^{-2} in 2007, and 2.70 ± 1.75 mg m^{-2} in 2012), but vertical distribution differed significantly. In 2007, the majority (62%) of integrated Chl *a* was located within the bottom 0.10 m of the ice, whereas in 2012 only 28% of Chl *a* was located in the lower-most 0.10 m (Fig. 3.2a, Appendix A). Absolute Chl *a* concentrations in the bottom 0.10 m of the sea ice were higher in 2007 (17.61 ± 21.27 μg L^{-1}) than 2012 (4.10 ± 5.42 μg L^{-1}), although the difference was not statistically significant (Fig. 3.2b, $p = 0.06$, Kruskal-Wallis test). The mean under-ice water Chl *a* concentration in 2012 (0.16 ± 0.07 μg L^{-1}) was significantly higher than in 2007 (0.05 ± 0.03 μg L^{-1}, $p < 0.01$, Kruskal-Wallis test, Fig. 3.2c).

3.4.2 Carbonate and lipid correction for zooplankton stable isotope values

After correction using equation (1), mean $\delta^{13}C$ values of the pteropod *L. helicina* were -25.41‰ and -24.44‰ for 2007 and 2012 samples, respectively, which were approximately 0.9‰ lower than the raw $\delta^{13}C$ values (Table 3.1).

Lipid-corrected $\delta^{13}C$ values were calculated using two generalised and two krill-specific mathematical models developed by previous studies (Table 3.1). For *L. helicina*, both raw and calcium carbonate-corrected $\delta^{13}C$ values were used in the calculations. $\delta^{13}C$ increased for all species following lipid-correction (Table 3.1, Fig. 3.3), but only lipid-rich *Clione limacina antarctica* showed a significant increase in lipid-corrected $\delta^{13}C$ calculated with model 1(Post et al., 2007, $p < 0.01$, two-way ANOVA). For species with C:N over 5 (*Primno macropa*, *Euchaeta antarctica*, *Archiconchoccia* spp., and *C. limacina antarctica*), model 2 (Logan et al., 2008) was more conservative than model 1, predicting a smaller lipid effect on $\delta^{13}C$ (Fig. 3.3). For species with a low lipid content

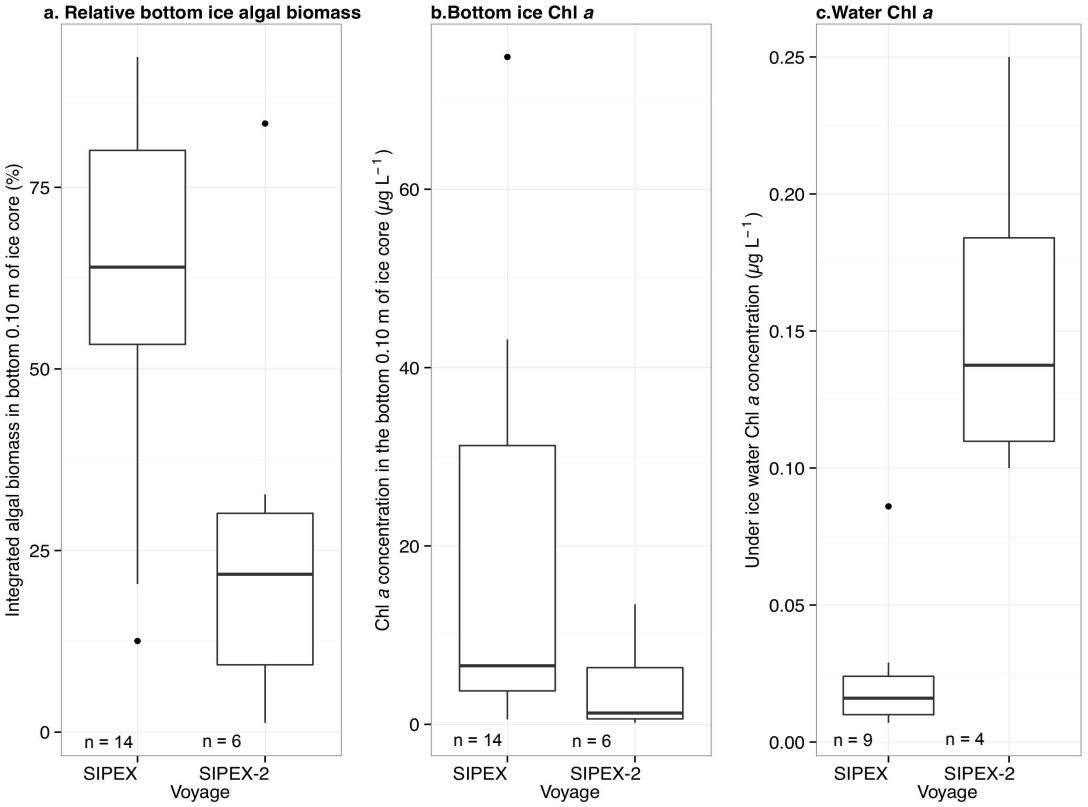

FIGURE 3.2: A comparison between SIPEX (2007) and SIPEX-2 (2012) of **a.** Percentage of integrated Chlorophyll *a* (Chl *a*) concentration in the bottom 0.10 m of sampled ice cores; **b.** Chl *a* concentrations (μg L^{-1}) in the bottom 0.10 m of sampled ice cores; **c.** Under-ice Chl *a* concentrations (μg L^{-1}) off East Antarctica during winter-spring transition. The line inside each boxplot represents the medium value. The boxes indicate the upper and lower quartiles, and lines outside boxes show total range of the data. Dots denote potential outliers.

(C:N $<$ 5), both generalised models were similar in performance, where model 1 and model 2 increased δ^{13}C on average by 0.7‰ and 0.6‰, respectively. Two krill-specific models resulted in larger increases in corrected δ^{13}C values in comparison with both generalised models (Fig. 3.3), but the differences among models were not significant (all $p > 0.5$, ANOVA).

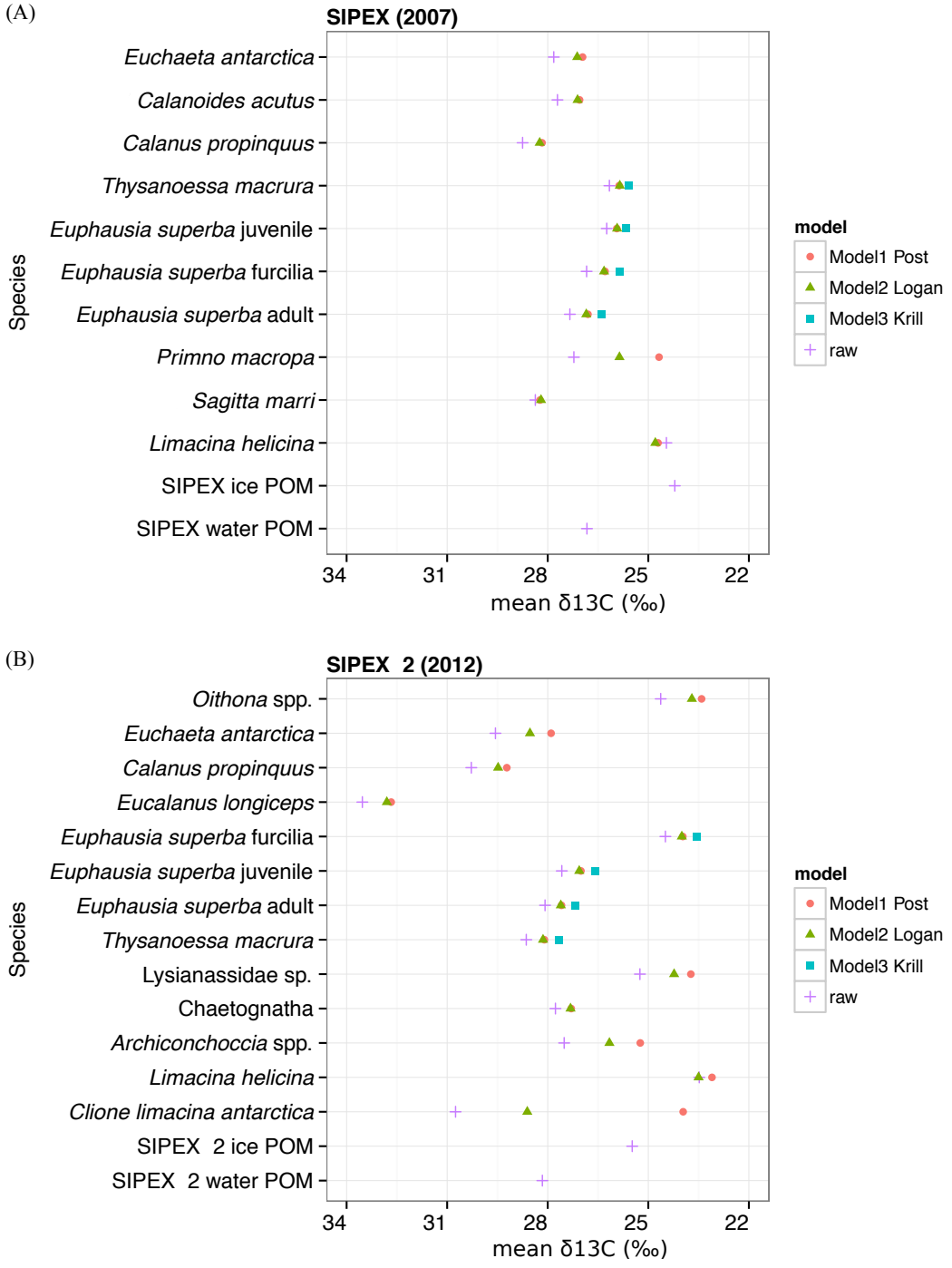

FIGURE 3.3: Comparison of mean raw and lipid-corrected δ^{13}C values in particulate organic matter (POM) and zooplankton sampled in **a.** SIPEX 2007 and **b.** SIPEX-2 2012 off East Antarctica during winter-spring transition. Filled red circles denote $\delta^{13}C_{lipid-corrected}$ from model 1 (Post et al., 2007), green triangles denote $\delta^{13}C_{lipid-corrected}$ from model 2 (Logan et al., 2008), blue squares denote $\delta^{13}C_{lipid-corrected}$ for krill species from krill-specific model 1(Logan et al., 2008), and crosses denote raw δ^{13}C. Species are listed according to the values of the mean $\delta^{13}C_{lipid-corrected}$ from model 1, within taxonomic class. Model details refer to main text and Table 3.1.

TABLE 3.1: A comparison between analysed δ^{13}C values and lipid-corrected δ^{13}C values for zooplankton samples collected during late winter/early spring off East Antarctica during two voyages SIPEX (2007) and SIPEX-2 (2012). Lipid-corrected δ^{13}C values were calculated with two generalised equations and two krill-specific equations. For *Limacina helicina*, both raw and carbonate-corrected δ^{13}C values were included in lipid calculations, and $\delta^{13}C_{lipid-corrected}$ values were listed in the ( ).

Species	Raw δ^{13}C (‰)	C:N	Lipid corrected δ^{13}C (‰)			
			Model 1[1]	Model 2 [2]	Krill-specific model 1 [3]	Krill-specific model 2 [4]
SIPEX 2007						
Calanoides acutus	-27.70	4.0	-27.05	-27.11	-	-
Calanus propinquus	-28.75	3.9	-28.17	-28.24	-	-
Euchaeta antarctica	-27.82	4.2	-26.96	-27.12	-	-
Euphausia superba adult	-27.34	3.9	-26.79	-26.85	-26.39	-26.39
Euphausia superba juvenile	-26.24	3.6	-25.94	-25.93	-25.66	-25.66
Euphausia superba furcilia	-26.84	3.9	-26.29	-26.33	-25.85	-25.84
Thysanoessa macrura	-26.16	3.6	-25.87	-25.85	-25.58	-25.58
Primno macropa	-27.22	5.9	-24.68	-25.86	-	-
Sagitta marri	-28.37	3.9	-28.23	-28.20	-	-
Limacina helicina	-24.47 (-25.41)	4.1	-23.76 (-24.71)	-23.84 (-24.79)	-	-
SIPEX-2 2012						
Calanus propinquus	-30.28	4.4	-29.22	-29.48	-	-
Euchaeta antarctica	-29.56	5.0	-27.90	-28.53	-	-
Eucalanus longiceps	-33.53	4.2	-32.67	-32.80	-	-
Oithona spp.	-24.63	4.6	-21	-23.70	-	-
Euphausia superba adult	-28.08	3.9	-27.59	-27.61	-27.18	-27.18
Euphausia superba juvenile	-27.58	3.9	-27.01	-27.06	-26.58	-26.58

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Table 3.1 – continued from previous page

Species	Raw δ^{13}C (‰)	C:N	Lipid corrected δ^{13}C (‰)			
			Model 1[1]	Model 2 [2]	Krill-specific model 1 [3]	Krill-specific model 2 [4]
Euphausia superba furcilia	-24.50	3.9	-23.97	-24.00	-23.55	-23.55
Thysanoessa macrura	-28.64	3.9	-28.10	-28.14	-27.66	-27.66
Lysianassidae sp.	-25.25	4.9	-23.73	-24.22	-	-
Chaetognatha	-27.77	3.8	-27.30	-27.32	-	-
Archiconchoccia spp.	-27.51	5.6	-25.24	-26.16	-	-
Limacina helicina	-28 (-24.44)	4.7	-22.14 (-23.10)	-22.55 (-23.50)	-	-
Clione limacina antarctica	-30.75	10.2	-23.96	-28.61	-	-

[1]Model 1, developed for aquatic animals Post et al. (2007):

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C - 3.32 + 0.99 \times C:N$$

[2]Model 2, developed for aquatic invertebrates (Equation developed in Fry (2002), and parameters were estimated by Logan et al. (2008)):

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 3.388 - \frac{3.388 \times 3.314}{C:N}$$

[3]Krill-specific model 1, (Equation developed in McConnaughey and McRoy (1979), and parameters were estimated for euphausiid species by Logan et al. (2008)):

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 3.713 \times (-0.051 + \frac{3.90}{1 + \frac{287}{L}})$$

$$L = \frac{93}{1 + (0.246 \times C:N - 0.775)^{-1}}$$

[4]Krill-specific model 2, (Developed in Fry (2002), and parameters were estimated for euphausiid species by Logan et al. (2008)):

$$\delta^{13}C_{lipid-corrected} = \delta^{13}C + 6.941 - \frac{6.941 \times 3.346}{C:N}$$

3.4.3 Stable isotope compositions of food web baselines: water POM and sea-ice POM

In both years, sea-ice derived POM and water column POM were isotopically distinguishable as two distinct carbon sources. Within each year, sea-ice POM had significantly higher δ^{13}C than that of water POM, but no significant interannual difference was found within each source (Table 3.2, $p < 0.01$, ANOVA followed by Tukey's Post Hoc test). During 2007, sea-ice POM had δ^{13}C values ranging from -27.13 to -21.11‰, and water POM ranging from -28.86 to -24.83‰. In 2012 both carbon sources had slightly lower δ^{13}C, with sea-ice POM ranging from -28.77 to -22.86‰ and water POM ranging from -30.54 to -26.63‰. Similarly, δ^{15}N in sea-ice POM showed a higher mean value than water column POM in 2007 (-4.91‰ and -8.35‰, respectively). In 2012, sea-ice POM had a mean δ^{15}N value of 4.3‰. No δ^{15}N values were obtained for water POM during that year.

TABLE 3.2: Stable isotope values (mean ± SD) and mean C:N ratios of particulate organic matter (POM) and zooplankton species collected during late winter/early spring off East Antarctica during two voyages SIPEX (2007) and SIPEX-2 (2012). N is the total number of samples analysed across the sampling area. δ^{13}C of water and sea-ice POM are raw values. δ^{13}C of zooplankton are lipid-corrected values using mathematic equation (Post et al., 2007; Logan et al., 2008, see Table 1 for detail). δ^{13}C values were corrected with the krill- specific model 1 for krill species (*Euphausia superba* and *Thysanoessa macrura*), and with model 1 (Post et al., 2007) for non-euphausiid species. Student-Newman-Keuls range test was used to compare mean δ^{13}C values of zooplankton within the year. Same test was also applied to mean δ^{15}N values of zooplankton within the year. Different letters in homogenous groups indicate the difference was significant, whereas the same letter or letter combination indicates non-significant difference ($p < 0.05$, Student-Newman-Keuls range test).

	n	δ^{13}C mean ± SD (‰)	Homog. groups	δ^{15}N mean ± SD (‰)	Homog. groups	C:N
SIPEX 2007						
Primary producers						
Water POM	12	-26.83 ± 1.31		-8.24 ± 6.48		
Sea ice POM	7	-24.21 ± 1.88		-4.91 ± 2.43		
Copepod						
Calanoides acutus	1	-27.05	bc	4.58	b	4.0
Calanus propinquus	22	-28.17 ± 1.06	c	1.46 ± 1.84	b	3.9
Euchaeta antarctica	3	-26.96 ± 1.38	bc	11.65 ± 1.93	a	4.2
Krill						
Euphausia superba adult	4	-26.39 ± 0.66	ab	2.84 ± 0.33	b	3.9

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Table 3.2 – continued from previous page

	n	δ^{13}C mean ± SD (‰)	Homog. groups	δ^{15}N mean ± SD (‰)	Homog. groups	C:N
Euphausia superba juvenile	5	-25.66 ± 0.23	ab	3.14 ± 0.23	b	3.6
Euphausia superba furcilia	5	-25.85 ± 0.38	ab	5.65 ± 0.56	b	3.9
Thysanoessa macrura	13	-25.58 ± 0.55	ab	5.31 ± 0.64	b	3.6
Amphipod						
Primno macropa	4	-24.68 ± 0.42	a	3.31 ± 1.64	b	5.9
Chaetognatha						
Sagitta marri	34	-28.23 ± 0.34	c	12.36 ± 2.20	a	3.5
Pteropod						
Limacina helicina	4	-24.71 ± 0.26	a	3.66 ± 0.98	b	4.1
SIPEX-2 2012						
Primary producers						
Water POM	12	-28.16 ± 1.31		–		
Sea ice POM	14	-25.48± 1.75		4.34 ± 0.91 (n=4)		
Copepod						
Calanus propinquus	18	-29.22 ± 0.97	c	5.11 ± 0.72	cde	4.4
Euchaeta antarctica	6	-27.90 ± 1.41	bc	7.20 ± 0.82	ab	5.0
Eucalanus longiceps	1	-32.67	d	3.37	e	4.2
Oithona spp.	3	-21 ± 0.26	a	6.41 ± 0.82	bc	4.6
Krill						
Euphausia superba adult	3	-27.18 ± 0.33	bc	5.10 ± 1.45	cde	3.9
Euphausia superba juvenile	16	-26.58 ± 1.05	abc	3.60 ± 0.44	e	3.9
Euphausia superba furcilia	6	-23.55 ± 1.20	a	4.44 ± 0.85	de	3.9
Thysanoessa macrura	20	-27.66 ± 0.82	bc	6.26 ± 0.96	bcd	3.9
Amphipod						
Lysianassidae sp.	2	-23.73 ± 2.98	a	7.89 ± 1.15	ab	4.9
Chaetognatha	4	-27.30 ± 0.55	bc	8.39 ± 0.65	a	3.8
Ostracod						

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Table 3.2 – continued from previous page

	n	δ^{13}C mean ± SD (‰)	Homog. groups	δ^{15}N mean ± SD (‰)	Homog. groups	C:N
Archiconchoccia spp.	3	-25.24 ± 1.10	b	6.49 ± 1.19	bc	5.6
Pteropod						
Limacina helicina	3	-23.10 ± 4.10	a	6.83 ± 1.61	abc	4.7
Clione limacina antarctica	3	-23.96 ± 5.35	a	5.10 ± 0.92	cde	10.2

3.4.4 Differences in stable isotope compositions among zooplankton species

Model corrected stable carbon isotope Of all eight zooplankton species analysed in 2007, the pteropod *L. helicina* and the amphipod *P. macropa* were enriched in ^{13}C, with δ^{13}C values similar to that for the sea-ice POM (mean: -24.46‰, Table 3.2, Fig. 3.4a). Both krill species, *E. superba* and *T. macrura*, had δ^{13}C signatures between that of water POM and sea-ice POM. Furcilia larvae and juvenile *E. superba*, and *T. macrura* had similar δ^{13}C values (mean: -25.85‰, -25.66‰, and -25.58‰ respectively), which were slightly more positive than adult *E. superba* (-26.39‰). The three copepod species (*Calanoid acutus*, *Calanus propinquus*, *E. antarctica*) had similar δ^{13}C values, and were slightly less positive than the mean water POM δ^{13}C (Table 3.2, Fig. 3.4a). Among those, *C. propinquus* had the lowest value (mean: -28.17‰). The chaetognath *Sagitta marri* had the lowest mean δ^{13}C ratio (mean: -28.23‰) among all species in 2007. Large variations were found in δ^{13}C values of *C. propinquus* and *E. antarctica* (n = 3).

Among the 11 zooplankton species analysed in 2012, five species had relatively high δ^{13}C values that were similar to sea-ice POM (Table 3.2, Fig. 3.4b). The pteropod *L. helicina* and *C. limacina antarctica* had high mean δ^{13}C (mean: -23.10‰ and -23.96‰, respectively), although with large variability (n = 3, respectively). Similar high δ^{13}C values were found for the small copepods *Oithona* spp., *E. superba* furcilia and amphipods from the family Lysianassidae (Table 3.2). In contrast to the high mean δ^{13}C of larval *E. superba*, lower mean δ^{13}C values were found for adult and juvenile *E. superba*, which were -27.18‰ and -26.58‰, respectively (Fig. 3.4b). Similarly, *T. macrura* also showed a lower δ^{13}C ratio (mean: -27.66‰, Table 3.2). Ostracods (*Archiconchoccia* spp, mean: -25.24‰) had a δ^{13}C value similar to those for the sea-ice POM. Chaetognaths had low

δ^{13}C ratios (mean: -27.30‰) that were slightly less positive than POM from the water column. The large copepods (*Eucalanus longiceps*, *C. propinquus*, *E. antarctica*) all appeared to be depleted in ^{13}C compared to *Oithona* spp.. *E. antarctica* and *C. propinquus* which had mean δ^{13}C values of -27.90‰ and -29.22‰, respectively. *Eucalanus longiceps* showed the lowest δ^{13}C value (mean: -32.67‰) among all zooplankton species analysed in 2012.

The mean δ^{13}C values of both sea-ice POM and water POM were approximately 1‰lower in 2012 compared to 2007 (Table 3.2). In contrast to the lower δ^{13}C values in food web baselines, larval *E. superba* had a significantly higher δ^{13}C in 2012 compared to 2007 (Table 3.2, $p < 0.01$, one-way ANOVA). Zooplankton analysed in 2012 generally showed larger variations in δ^{13}C values compared to those analysed in 2007 (Table 3.2).

Stable nitrogen isotope In 2007, the δ^{15}N values showed a clear stepwise increase within the range of 1.46‰ in copepods to 12.36‰ in chaetognaths (Fig. 3.5a). The copepod *C. propinquus* had the lowest δ^{15}N value (mean: 1.46‰) of the eight zooplankton species analysed (Table 3.2, Fig. 3.5a). Adult and juvenile *E. superba*, *P. macropa*, and *L. helicina* had similar δ^{15}N values at ∼3‰, which were about 1.5‰ higher than *C. propinquus*. The copepod *C. acutus* had a slightly higher δ^{15}N at 4.58‰. *Thysanoessa macrura* and larval *E. superba* shared similar δ^{15}N values at ∼5.5‰, and these were ∼4‰ higher than *C. propinquus*. The carnivorous copepod *E. antarctica* and the chaetognath *S. marri* had the highest δ^{15}N values, with values reaching over 11‰, and were ∼10‰ higher than *C. propinquus*.

In 2012, δ^{15}N values showed a continuous rather than stepwise increase from 3.37‰ in copepods to 8.39‰ in chaetognaths. The δ^{15}N values showed considerable overlap among species (Fig. 3.5b). The lowest δ^{15}N values were found in the copepod *E. longiceps* (mean: 3.37‰) and juvenile *E. superba* (mean: 3.60‰), followed by larval *E. superba* (mean: 4.44‰). Adult *E. superba*, *C. limacina antarctica* and *C. propinquus* had similar δ^{15}N values at ∼5‰. *Thysanoessa macrura*, *Oithona* spp., *Archiconchoccia* spp., and *L. helicina* were slightly higher in δ^{15}N at ∼6‰. Amphipods (mean: 7.89‰), the chaetognaths (mean: 8.39‰), and the calanoid copepod *E. antarctica* (mean: 7.20‰) had the highest δ^{15}N values, with all exceeding 7‰.

Stable isotope mixing model We performed SIAR models to estimate the proportional dietary contribution of pelagic and sea-ice POM to zooplankton species. Three sets of trophic fractionation factors were adopted. The estimated contributions of both food sources were strongly affected by the set of values we chose (Appendix B, FigB.1). Within each case, the estimation showed considerable variation (Appendix B, Table B.1).

3.5 Discussion

3.5.1 Carbonate and lipid effects on zooplankton δ^{13}C values

Inorganic carbonate and lipid content in organisms can affect stable isotopic ratios, and thus can lead to false interpretation of dietary or habitat shfits (DeNiro and Epstein, 1977; Logan et al., 2008; Pomerleau et al., 2014). Inorganic carbon is incorporated into organisms from different sources, and tends to have higher δ^{13}C values compared to

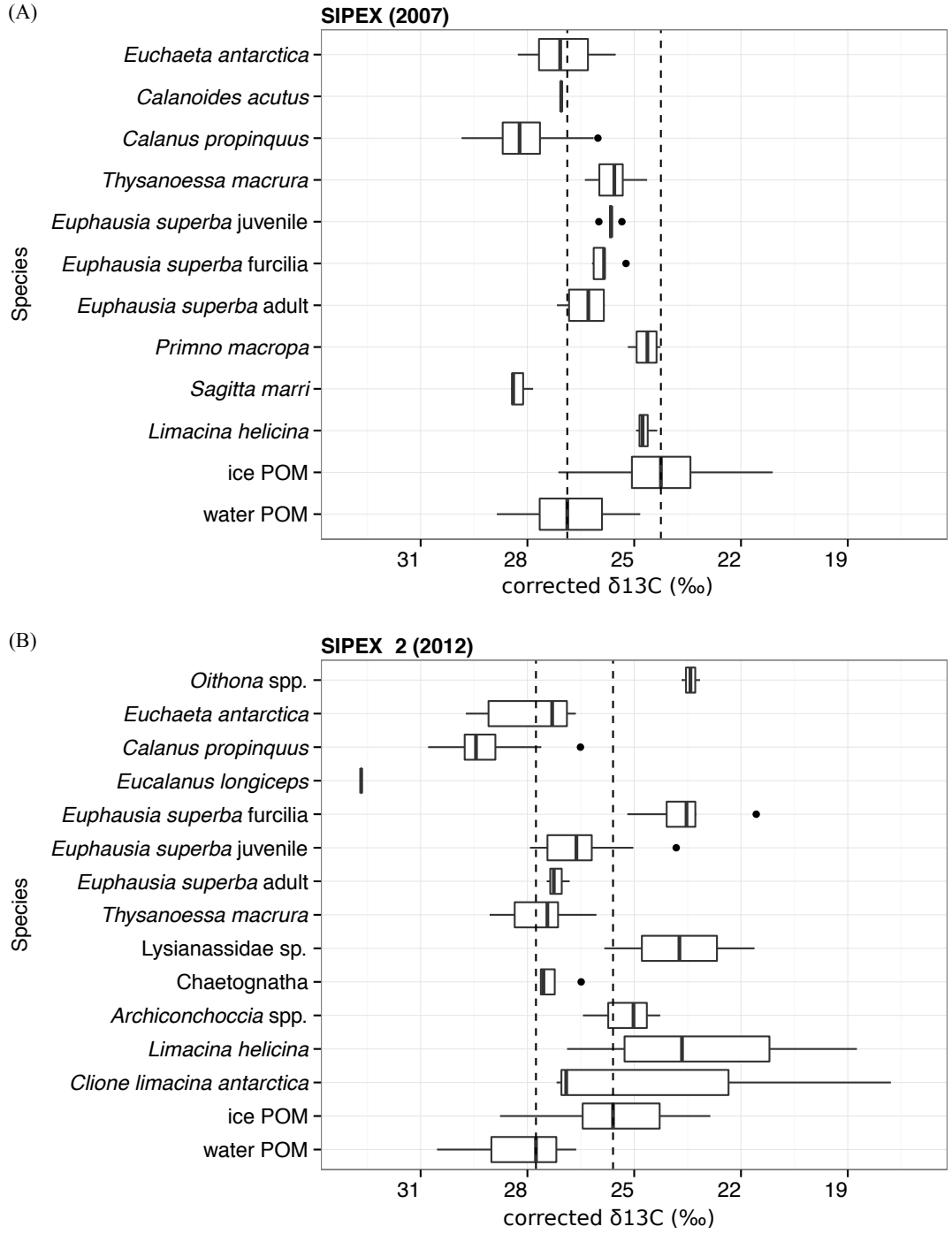

FIGURE 3.4: Raw δ^{13}C values (‰) in particulate organic matter (POM) and zooplankton sampled in **a**. SIPEX (2007), **b**. SIPEX-2 (2012) off East Antarctica during winter-spring transition. The dashed lines in a. and b. denote the medium δ^{13}C values of water POM and sea-ice POM. For details of the boxplot representation, see Fig.2. Species are listed according to the value of the mean $\delta^{13}C_{lipid-corrected}$, within taxonomic class.

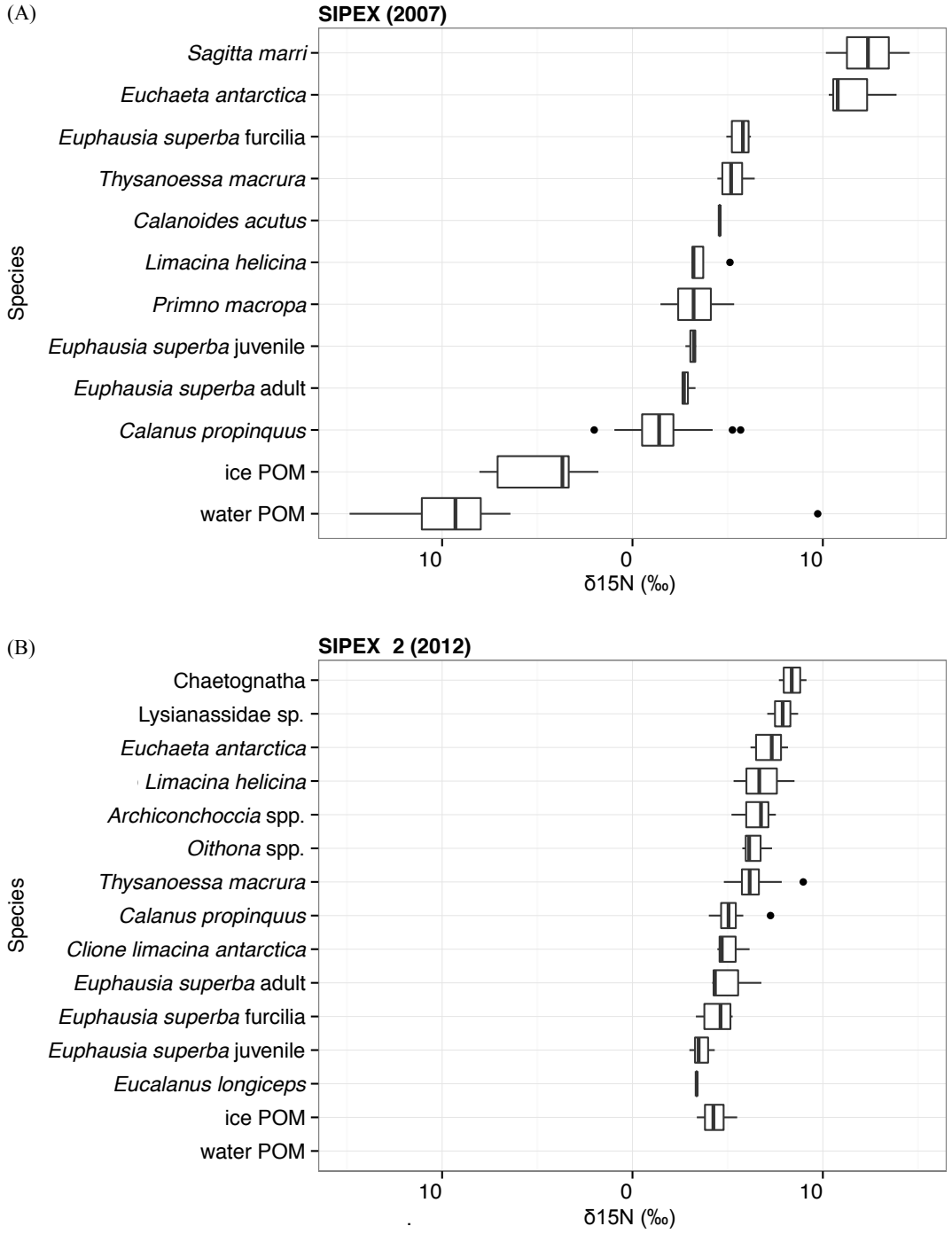

FIGURE 3.5: δ^{15}N values (‰) in particulate organic matter (POM) and zooplankton sampled in **a.** SIPEX (2007), **b.** SIPEX-2 (2012) off East Antarctica during winter-spring transition. For details of the boxplot presentation, see Fig.2. Species are listed according to the mean δ^{15}N values.

organic carbon (Jacob et al., 2005; Pomerleau et al., 2014). Lipids have more negative δ^{13}C values relative to other biochemical compounds (Post et al., 2007; Logan et al., 2008). Therefore, carbonate- and lipid-correction for δ^{13}C values are believed to be important for stable isotope studies. However, methods of both acidification and lipid extraction are as yet not fully standardised and it remains unclear how the extraction prior to the stable isotope analysis will affect δ^{15}N values (Jacob et al., 2005; Post et al., 2007; Logan et al., 2008; Mintenbeck et al., 2008). A separate set of untreated samples is needed for δ^{15}N analysis if lipids are extracted with chemical methods (Stowasser et al., 2012). The more cost-effective way for sample correction is through mathematical corrections after the analysis (Logan et al., 2008), which was adopted in our study to calculate both carbonate and lipid effects.

Carbonate-correction was only applied to δ^{13}C values for the shelled pteropod *L. helicina*, as suggested in Pomerleau et al. (2014). Both corrected and raw δ^{13}C values suggested *L. helicina* relied heavily on ice biota during both of our sampling years.

For lipid correction, although both generalised models predicted similar results for lipid-poor species, model 1 seemed to perform better with lipid-rich materials than model 2 (Table 3.1, Fig. 3.3). Therefore, model 1 (Post et al., 2007) was adopted in our study for lipid correction of non-euphausiid species. Logan et al. (2008) have pointed out that species-specific models should be used for lipid-correction if available. As the two krill-specific models compared in our study produced nearly identical results, we adopted the result from krill-specific model 1 for the krill species. It should be highlighted that the choice of lipid-correction models could lead to different ecological interpretations. Because a widely accepted correction model is not yet available, it is important to report raw δ^{13}C values to enable cross-study comparison.

3.5.2 Food web baseline - Particulate Organic Matter

We found higher δ^{13}C values in sea-ice POM compared to water column POM, which is consistent with previous studies on Arctic and Antarctic sea ice (Rau et al., 1991b; Hobson et al., 1995; Kennedy et al., 2002). The higher δ^{13}C values in sea-ice POM are likely a result of low CO_2 availability in the sea ice (Rau et al., 1991b; Francois et al., 1993; Laws et al., 1995). The mean δ^{13}C of both sea-ice and water POM in 2012 were lower than 2007 samples by ~1‰ (Table 3.1, Fig. 3.4), additionally highlighting the natural variation between sampling years.

POM from both the sea ice and the water column showed low negative δ^{15}N values in 2007, which were lower than any previous records from pack ice and surface water in the Southern Ocean (Rau et al., 1991b; Fripiat et al., 2014). As the possibility of

technical contamination was ruled out, we suggest this observed low δ^{15}N in sea ice is because of ammonium-dominated metabolism (Kristiansen et al., 1992; Trull et al., 2008; Sigman et al., 2009; Trull et al., 2014). In 2007, colder sea ice with a lower brine volume fraction potentially reduced the ice permeability and limited the supply of external "new" nutrients, which resulted in increased regenerated production in sea ice. Elevated ammonium (NH_4^+) concentrations were found in sea ice bottom sections in 2007 (Meiners et al., 2011) compared to 2012 (Ugalde et al., this issue). The low δ^{15}N of seawater POM also suggested ammonium recycling and uptake during algal growth, which has previously been reported from Antarctic surface water (Probyn and Painting, 1985; Smith and Nelson, 1990; Trull et al., 2014).

In 2012, sea-ice POM had a mean δ^{15}N value of 4.34‰, which reflected nitrate (NO_3^-) assimilation within the sea ice (Fripiat et al., 2014). As the ice conditions were remarkably different between years, the interannual difference in δ^{15}N might be a reflection of δ^{15}N seasonal change in sea ice. It is possible that ice algal metabolism was ammonium-dominated during late winter under a low temperature regime, but increasing temperatures may have increased both interior brine convection and brine-seawater exchange towards early spring, which introduced nitrate from seawater (Griewank and Notz, 2013). The increase in nitrate consumption led to enrichment of ^{15}N in sea-ice POM. The increased brine-seawater exchange also made sea-ice biota more accessible for zooplankton in the water column in early spring, which was reflected in our δ^{13}C results as discussed below.

3.5.3 Zooplankton dietary difference between years

Carbon isotopic values in consumers reflect their diet (McCutchan et al., 2003). As δ^{13}C values of both sea-ice and water POM decreased in 2012, animals having the same diet between 2007 and 2012 would be expected to show depletion in ^{13}C in 2012. This was observed in most of the species we sampled except larval *E. superba*, amphipods, chaetognaths, and *L. helicina*. Also, animals generally showed higher variance in δ^{13}C and δ^{15}N in 2012, which suggested animals in this year were either feeding more opportunistically or consumed a more varied diet (Bearhop et al., 2004; Sweeting et al., 2005).

3.5.3.1 *E. superba*

The significant increase in δ^{13}C of larval *E. superba* clearly indicated a dietary shift towards a sea-ice food source from 2007 to 2012. Warmer ice with more brine in 2012 might have made the ice biota more accessible to furcilia larval *E. superba* under the

ice, which might explain their interannual dietary variability. It is worth noting that, in 2007, larval *E. superba* consuming a mix of pelagic- and sea-ice-based food had higher mean δ^{15}N value (5.65‰), which was similar to that of the known omnivorous krill *T. macrura* (5.31‰). This suggested a heterotrophic diet for larval *E. superba* in 2007. The high abundance of tintinnids in the water column reported from the same voyage (Wallis et al., this issue) provided potential dietary source for *E. superba* larvae. Lipid analysis indicated that in 2007, *E. superba* furcilia larvae were in good condition (O'Brien et al., 2011). The same study reported a higher ratio between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) in these larval *E. superba*, which has been interpreted as an index of carnivory for krill in good condition (Cripps et al., 1999; Cripps and Atkinson, 2000). Copepod fatty acid biomarkers found in these *E. superba* furcilia larvae also suggested a heterotrophic diet (O'Brien et al., 2011). Although krill larvae have been widely recognised as being associated with sea ice as it is an important refuge from strong currents and predators, as well as a feeding ground (Garrison, 1991b; Brierley et al., 2002; Meyer et al., 2009; Meyer, 2012), it remains an open question whether sea-ice biota is a main dietary source for krill over winter. Our results suggest that krill larvae from our sampling region did not necessarily rely on sea-ice biota, but also consumed a heterotrophic diet sourced from the water column to support their energy needs.

3.5.3.2 Amphipods

Different species of amphipods were sampled during the two voyages, possibly resulting in increases in δ^{13}C values from 2007 to 2012. The hyperiid amphipod *P. macropa* (-24.68‰) and the gammarid species (Family Lysianassidae, -23.73‰) were sampled in 2007 and 2012, respectively. Amphipods dominate Arctic under-ice communities, but little is known about them under Antarctic sea ice. In areas where they are common, amphipods are likely to have a great impact on sea-ice primary production (Arndt and Swadling, 2006). In our study, both species had high $\delta^{13}C_{lipid-corrected}$ signals, suggesting a sea-ice POM based diet. Previous studies have found both *P. macropa* and gammarid amphipods winter sea ice; gammarid amphipods in particular were found closely associated with sea ice (Kaufmann et al., 1993, 1995; Arndt and Swadling, 2006; Krapp et al., 2008; Flores et al., 2011; Hunt et al., 2011). Our $\delta^{13}C_{lipid-corrected}$ values for the gammarid amphipod agree with the hypothesis that these crustaceans are able to exploit under-ice habitats (Flores et al., 2011; Swadling, 2014), but we were unable to estimate whether gammarid amphipods were a substantial component in the under-ice zooplankton community due to the limited number of specimens collected.

3.5.3.3 Chaetognaths

In 2007 the sampled chaetognath species was *S. marri*, whereas the chaetognaths caught in 2012 were likely to be *Eukrohnia* spp. (Wallis et al., this issue). Dietary differences between species might be the reason for a small shift in δ^{13}C from 2007 to 2012. In both years, chaetognath species had the highest δ^{15}N among all zooplankton in our study, which agreed with the widely accepted idea that chaetognath species are pelagic predators (Øresland, 1990; Froneman and Pakhomov, 1998; Grigor et al., 2014). The low $\delta^{13}C_{lipid-corrected}$ suggested *S. marri* found in our study ingest a pelagic-based diet. On the other hand, chaetognaths in 2012 were likely to consume a mixed diet from both the sea ice and the water column, although the main food source was probably from the water column. This is consistent with the finding of Flores et al. (2014) that high abundances of chaetognaths in the genus *Eukrohnia* occurred in the surface water under the Antarctic winter sea ice, with a strong diel vertical migration.

3.5.3.4 Pteropods

The internal shift in δ^{13}C of *L. helicina* suggested increased dietary contribution from sea ice for this species in 2012. Gannefors et al. (2005) reported that Arctic *L. helicina* rely on sea-ice POM in winter, but little is known about the survival of Antarctic *L. helicina* in ice-covered areas during winter. Recent studies have reported high density of *L. helicina* under winter sea ice (Flores et al., 2011; Hunt et al., 2011; Flores et al., 2014), and our stable isotope results indicated that *L. helicina* in Antarctica, similar to their northern relatives, also rely on sea-ice POM in winter.

Clione limacina antarctica was only present in our 2012 samples. Previous studies concluded that *C. limacina antarctica* fed exclusively on *L. helicina* (Gilmer and Lalli, 1990; Bryan et al., 1995), and the finding of their increased association with sea ice also suggested this species adopts hibernal feeding on an ice-based diet (Flores et al., 2011, 2014). Our $\delta^{13}C_{lipid-corrected}$ clearly supported this food chain: ice algae $\rightarrow$ *L. helicina* $\rightarrow$ *C. limacina antarctica*. However, we did not find expected ^{15}N enrichment in *C. limacina antarctica*, δ^{15}N values of which were close to their prey *L. helicina*. One possible reason for the low δ^{15}N values is the potential effect of high lipid content of *C. limacina antarctica*. Usually lipid was believed to have a very small effect on zooplankton δ^{15}N values. However, zooplankton rarely have C:N over 10. It has been reported that lipid-rich materials are low in δ^{15}N (C:N >10, Sweeting et al., 2006; Bodin et al., 2007). In our study, *C. limacina antarctica* had the highest C:N ratio at 10.2 among all analysed species. Further studies on lipid-rich zooplankton species are needed to quantify and confirm this lipid effect on δ^{15}N values.

3.5.4 Trophic structure and stable isotope mixing model

δ^{15}N values are generally used as an indicator for trophic positions (Minagawa and Wada, 1984; Peterson and Fry, 1987). Our δ^{15}N results in general reflected trophic positions, with lowest nitrogen isotopic values found for herbivorous copepods and the highest for chaetognaths. Previous stable isotope studies often calculate trophic levels based on δ^{15}N values so that trophic structures can be described quantitatively (e.g. Hobson et al., 1995; Stowasser et al., 2012). However, because two food web baselines (water POM and sea-ice POM) existed in the present study, precise calculation of trophic levels would not be available here without further understanding of the relative contributions of each food source.

Limited information was obtained from our SIAR models due to considerable variations in model estimates. This variation in the estimated proportional contribution could be due to the large variation and overlap in isotopic values between our two food web baselines. Filtering a range of particles together, to represent POM tends to 'average' the δ^{13}C and δ^{15}N contributions from different food sources. The fact that some copepod species analysed in this study showed lower δ^{13}C values than both pelagic and sea-ice POM might be the result of such an 'averaging' effect. Therefore, attempts to reduce variations in POM isotopic values, such as size fractionation during POM sampling, should be considered in the future.

The mixing model requires input of trophic enrichment factors for both δ^{15}N and δ^{13}C isotopes. For δ^{15}N, 3.4 ± 1.1‰ per trophic level is a generally accepted enrichment factor across a variety of ecosystems (Minagawa and Wada, 1984), but the reported δ^{13}C enrichment factors ranged from 0 to 1.5‰ per trophic level (McConnaughey and McRoy, 1979; Rau et al., 1983; Fry and Sherr, 1989). Although Rau et al. (1983) suggested that pelagic ecosystems have a larger δ^{13}C trophic enrichment factor (1.5‰; McConnaughey and McRoy, 1979) than lakes and coastal systems, this has not been proved. With the mixing model, we tested three sets of values for δ^{15}N and δ^{13}C, 3.4 ± 1‰ and 1.5‰ (McConnaughey and McRoy, 1979), 2.2 ± 0.3‰ and 0.5 ± 0.13‰ (McCutchan et al., 2003), and 3.4 ± 1‰ and 0.4 ± 1.3‰ (Post, 2002a), respectively. The estimated contributions of both food sources were strongly affected by the set of values we chose (Appendix B, Fig. B.1), and no further evidence could support a choice of either set of values for the food web in this study. Therefore, further calculation with SIAR requires a better understanding of trophic enrichments for δ^{15}N and δ^{13}C isotopes in Southern Ocean plankton.

3.5.5 Sea ice and the water column, which is the major nutrient source?

Our questions, concerning the contribution of sea-ice biota and pelagic biota and the trophic structure of the under-ice community, are challenging to address quantitatively with current knowledge. The stable isotope mixing model SIAR was applied for this purpose, but was of limited use without a better understanding of δ^{13}C and δ^{15}N trophic enrichment. The separation of δ^{13}C values through the mesozooplankton food web confirmed that both the sea ice and water column served as important dietary sources for the under-ice zooplankton community during the winter-spring transition. Sea ice was a major food supplier for the herbivorous pteropods and amphipods in both 2007 and 2012, as well as small copepods, ostracods and *E. superba* larvae in 2012 (Fig. 3.6). Predatory pteropods, which exclusively fed on the herbivorous pteropods, also indirectly rely on sea ice (Fig. 3.6). The comparison between years suggested that larval *E. superba* relied more on a heterotrophic diet when they did not feed solely from sea ice, a finding which supports earlier work (Huntley et al., 1994; Atkinson et al., 1999; Meyer et al., 2009; Schmidt et al., 2014). For the post-larval *E. superba* and omnivorous krill *T. macrura*, both sea-ice biota and pelagic biota possibly contributed to their diets, but adult *E. superba* showed a greater preference for a pelagic diet compared to juvenile *E. superba*. Herbivorous copepods tended to obtain food from the water column even when they lived under the ice cover. At the top of this food web, carnivores, including chaetognaths and copepods, showed carbon isotopic signals that were similar to the water POM.

In conclusion, our study described stable isotope compositions of zooplankton under pack ice during the winter-spring transition in East Antarctica and highlighted the dietary flexibility of larval *E. superba* during this food-limited season. Along with the well studied *E. superba* larvae, pteropods, amphipods and small copepods *Oithona* spp. also heavily relied on sea-ice biota. Post larval *E. superba* and *T. macrura* appeared to obtain food from both sea ice and the water column. Large copepods and chaetognaths, however, overwintered with a water column POM based diet. Our results suggest that the proportion of each food source varied with availability and amount of food in the sea ice. Warm and permeable sea ice is more likely to provide food for zooplankton species under the ice than colder (and less porous) ice, while the availability and amount of food in the water column during the winter-spring transition is overall less variable.

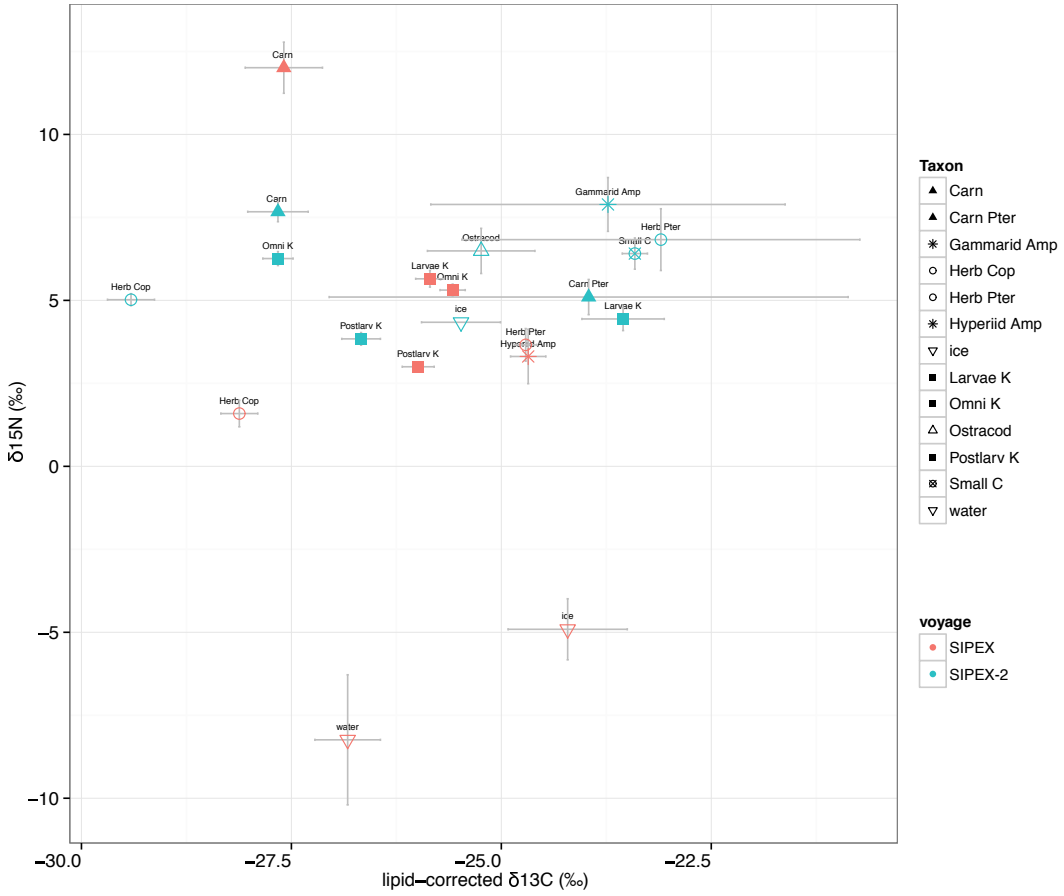

FIGURE 3.6: Relationship of lipid-corrected δ^{13}C and δ^{15}N values (mean ± standard error) of particulate organic matter (POM) and groups of zooplankton species off East Antarctica during winter-spring transition in 2007 (red) and 2012 (blue). ice = sea-ice POM; water = water POM; Herb Cop = Herbivorous copepods; Herb Pter = Herbivorous pteropods; Larvae K = Larval krill; Omni K = Omnivorous krill (*Thysanoessa macrura*); Postlarv K = Post-larval krill; Small C = Small Copepods; Hyperiid Amp = Hyperiid amphipods; Gammarid Amp = Gammarid amphipods; Carn = Carnivores; Carn Pter = Carnivorous pteropods.

Chapter 4

Using qualitative network models to test uncertainties regarding krill diet under winter sea ice

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4.1 Abstract

Winter is a critical season for the survival of Antarctic krill (*Euphausia superba*), yet evidence is conflicting about what krill feed on during winter. We use qualitative network models to evaluate different overwintering feeding scenarios for this species. By stepwise increasing the model complexity, we identify the necessary level of model complexity and capture the important processes driving krill dynamics. We highlight the need for stage-specific dietary information during food web model construction. Our approach captures that the relationship between protozoa and pelagic primary production is a key linkage governing the changes in krill population. These results demonstrate that qualitative network modelling approaches can be used for testing hypotheses about the structure and function of ecological systems, and for finding critical linkages for future research.

4.2 Introduction

Antarctic krill (*Euphausia superba*), a key species in Southern Ocean ecosystems, have historically been considered a pelagic herbivore. Increasing evidence indicates that Antarctic krill are not only an effective phytoplankton grazer, but also able to utilise other food sources, such as sea-ice biota (Marschall, 1988; Daly, 1990; Quetin et al., 1996; Frazer et al., 2002), copepods (Huntley et al., 1994), protozoans (Marchant and Murphy, 1994; Schmidt et al., 2006), and sea-bed detritus (Kawaguchi et al., 1986; Schmidt et al., 2011). Regional, seasonal, and ontogenetic variability in krill feeding have also been observed (Atkinson et al., 2002; Quetin and Ross, 2009; O'Brien et al., 2011; Schmidt et al., 2014). Despite our increasing knowledge of Antarctic krill feeding, dietary relationships of this species are generally over-simplified in ecosystem models (Murphy et al., 2012). Many Southern Ocean food web models (e.g. Mackey et al., 2012; Melbourne-Thomas et al., 2013) assume a dominant trophic pathway, where Antarctic krill is solely considered a pelagic diatom grazer (Laws, 1984). This relationship might be true under phytoplankton bloom conditions, however, alternative dietary relationships need to be considered in winter, when phytoplankton are scarce (Quetin and Ross, 2009).

Recent modelling studies have incorporated more complex dietary links for krill on regional scales (e.g. Hill et al., 2012; Murphy et al., 2013; Ballerini et al., 2014). Lowe et al. (2012) also incorporated diel vertical migration in krill feeding models. The complexity and observed regional variability in krill feeding (Schmidt et al., 2014), together with

the small number of regional samples, makes it difficult to develop a generalised understanding of krill diet (Atkinson et al., 2012a). Our understanding of krill winter feeding, in particular, has been most conflicting due to the scarcity of observations (reviewed in Meyer, 2012). Food in the water column is at the lowest level of the year during the austral winter. Larval krill rely on sea-ice biota as an alternative food source during this season (Quetin and Ross, 2009), however, dietary content of older krill changes with food availability (Schmidt et al., 2014). Sea ice conditions in Antarctica have been changing in recent decades (Stammerjohn et al., 2012; Massom et al., 2013), but predicting how these changes affect krill populations is not straightforward. The non-linear dietary relationships involved in sea-ice food webs feature indirect effects and system feedbacks, which are difficult to define with empirical evidence. Also, logistic difficulties make long-term or frequent measurements in sea-ice zones impossible, resulting in a large knowledge gap regarding sea-ice food webs and related ecosystem components. Inadequate data and limited mechanistic understanding of the ice-associated food web structures have restricted the development of quantitative food web models for Antarctic sea-ice ecosystems. Furthermore, the influence of model structure and complexity on model dynamics has rarely been discussed.

Unlike quantitative modelling approaches that require detailed information to parameterise individual model processes, qualitative modelling provides a general casual understanding of ecosystem dynamics based on on qualitative information about network structure (Dambacher et al., 2009). Qualitative network modelling is based on a set of assumptions about key ecosystem components and the sign of direct interactions between them (Raymond et al., 2010; Melbourne-Thomas et al., 2012). This approach is well suited to formulate and explore alternative ecosystem model structures, and to assess the implication of this structural uncertainty to model dynamics based on system feedback. Thus, qualitative network modelling is a valuable tool to test alternative assumptions about system structure and provide a general understanding of system functioning in data poor context, and/or to forerun more detailed quantitative models (Dambacher et al., 2007; Marzloff et al., 2011; Melbourne-Thomas et al., 2013).

In this study, we apply a qualitative network modelling approach to explore krill dietary relationships in winter. We evaluate the effects of increasing the food web complexity on krill responses to winter conditions. Different theories have been proposed for krill feeding overwinter, and here, we construct a series of alternative models of different levels of complexity that represent these theories. We then compare qualitative model outputs, and identify important linkages and variables that affect the dynamics of

krill populations. We test krill overwintering success under alternative feeding assumptions, evaluate a set of overwintering feeding scenarios for Antarctic krill, and suggest feeding strategies that are likely to sustain krill populations in winter. Our approach demonstrates the potential of using network analysis to evaluate assumptions about the structure of ecological systems, and priorities for future ecological research.

4.3 Materials and methods

4.3.1 Qualitative network approach

Network models are made up of variables and linkages. The analysis of network models is based on mathematical analysis of the community matrix. Qualitative network approach predicts the model response to a long-term perturbation by analysing the inverse community matrix, in which only the signs of matrix elements are specified (Levins, 1974). As only the signs of the matrix elements are specified, qualitative models can be described using a signed directed graph, also referred to as 'signed digraph'. The signed digraph represents our hypothesis about system structure. Variables represent the modelled populations (single species, functional groups, or environmental variables), shown graphically as nodes. Variables are connected by linkages that represent interaction coefficients in the community matrix, which describes the pairwise interactions between modelled populations ($\rightarrow$ for positive direct effect, or $-\bullet$ for negative direct effect). Model linkages can represent both trophic interactions (prey and predator) and other ecological interactions (i.e. competition, habitat dependencies, environmental drivers). In our study these digraphs were constructed with the drawing program Dia (http://live.gnome.org/Dia).

In our modelled system, the prey-predator relationship is not fully understood, and uncertainty remains regarding the strength of the interactions between modelled populations. This leads to uncertainty in model parameterisation. Our qualitative network approach suits this scenario, and is able to make qualitative predictions about the system responses with only knowledge of the system structure and the signs of the interactions (Melbourne-Thomas et al., 2012). This is done by simulating each model configuration for a number of times (n = 5000 in our case) using randomly assigned interaction weights (drawn from a uniform distribution). At each step, model stability is tested by examining the eigenvalues of this quantitatively specified community matrix, and only results from stable configurations are aggregated to provide estimates of the likelihood of different outcomes. The use of randomly assigned weights effectively allows the analyses

to explore the effects of parametric uncertainty. The analysis is conducted with QPress (R package, Melbourne-Thomas et al., 2012).

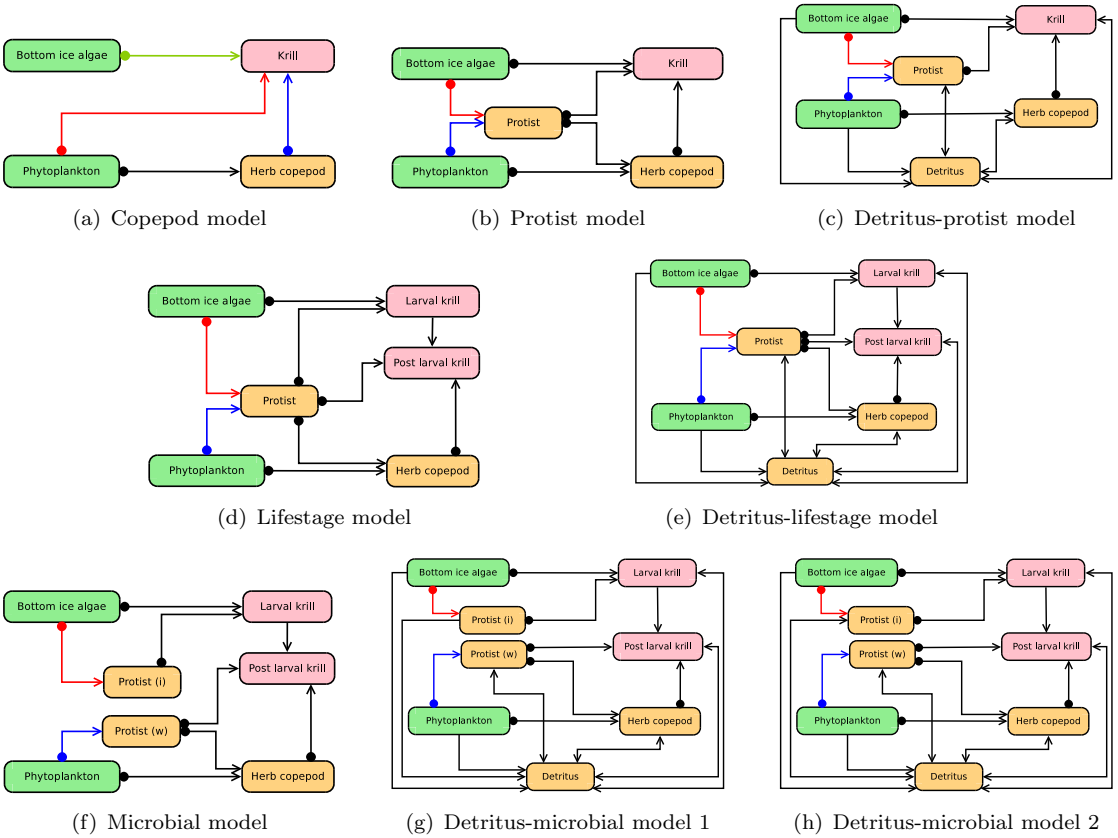
Our approach predicts the qualitative responses of system variables to 'press perturbation', i.e. a sustained change in the abundance or level of one or more components. The sign of the qualitative predictions can be fully determined, or ambiguous when both positive and negative effects contribute to a variable response. Here, we present model predictions as probabilities of a negative response. The blue-white-red colour scaling goes from blue, for a 0 probability, to red, for a 100% probability of a negative response (see figures in the results section). A low probability of a negative response, shown in dark blue, can be interpreted as a highly likely positive or neutral response. A 50% ($\pm$ 10%) probability of a negative response, shown in light colours, is considered an ambiguous response overall, as in this case it is unclear whether the most frequent model outcome is positive, negative or zero (no response). Highly likely negative responses are shown in dark red. This visualisation method has been developed by Marzloff and Melbourne-Thomas (unpublished). More detailed descriptions of the method are given in (Marzloff et al., submitted).

It is worth noting that our approach does not involve data for model construction or model evaluation. Alternatively, we directly compare qualitative outcomes between models to evaluate the effects of model structure and processes on modelled variables.

4.3.2 Model specification

We formulated eight sets of models to represent different krill dietary relationships under the sea ice during the winter/spring period (Appendix C and D) . Our models generally capture the dynamics of under-ice krill feeding, and thus they only include key system components (Fig. 4.1). Interactions were characterised in terms of both signs and certainty during model construction. Solid lines represented strong and certain interactions. Dashed lines represented uncertain interactions, of which the evidence for their importance is controversial (see Appendix C). Four variables were included in the basic model set (model A) for testing the linkage effects on model prediction. More variables were added stepwise to the basic model to increase the model resolution. Specifically, we included bottom sea-ice algae and under-ice phytoplankton as two carbon sources throughout all model configurations. Copepods, protists and detritus were added in the more complex models, and were considered as additional available preys for krill. We also presented the case where krill were considered as two separate life-stages, larval and

post-larval krill.



(a) Copepod model (b) Protist model (c) Detritus-protist model

(d) Lifestage model (e) Detritus-lifestage model

(f) Microbial model (g) Detritus-microbial model 1 (h) Detritus-microbial model 2

FIGURE 4.1: Qualitative network models for different Antarctic krill winter dietary relationships. Arrows and filled circles denote positive and negative effects, respectively. Coloured lines represent alternative model structures. Green nodes represent primary producers, pink nodes represent Antarctic krill, and yellow nodes represent other ecological components in the system. **a**. Copepod model; **b**. Protist model; **c**. Detritus-protist model; **d**. Lifestage model; **e**. Detritus-lifestage model; **f**. Microbial model; **g**. Detritus-microbial model 1; **h**. Detritus-microbial model 2. All model components have a self-limitation interaction (negative), but not shown in this figure. Model **d** has seven alternative model structures, detailed description is provided in the appendix C. Model **b** to **h** have four alternative models, respectively. Detailed description is given in Table 4.1 and the method section.

The basic model A (Fig. 4.1a), referred to as 'copepod' model, comprised four main components of sea-ice food webs. 1) 'Bottom ice algae' represents all species of microalgae inhabiting the bottom of the sea ice, which is available for under-ice grazers. 2) 'Phytoplankton' represents all sizes and species of pelagic phytoplankton in the under-ice water column. 3) Antarctic krill (krill) is considered as an individual variable. 4) Herbivorous copepods (herb copepod) represent suspension feeding copepod species in the water column. In model B, the protist model (Fig. 4.1b), 'protist is an additional variable that represents both mixotrophic and heterotrophic protozoans existing in both the water column and the sea ice. 'Detritus' was then added in the detritus model (Fig.

4.1c) to represent both pelagic detritus (i.e. marine snow) and sea-bed detritus. In two lifestage models (model D, Fig. 4.1d; model E, 4.1e), based on the protist and detritus model, respectively, the krill population was split into larval and post-larval stages to include stage-specific dietary information. Finally, 'protist' was specified as sea-ice protists (protist (i)) and pelagic protists (protist (w)) in three microbial models, models F, G, H (Fig. 4.1f, 4.1g, 4.1h). All model variables are self-limited (a negative effect from the variable to itself), but self-limitations are not shown in Fig. 4.1 for clarity of presentation.

The copepod model (model A, Fig. 4.1a) assumes that krill feed on sea-ice algae during winter (Quetin et al., 1996; Frazer et al., 2002). Additionally, krill also obtain nutrition over winter by feeding on under-ice phytoplankton and copepods (Huntley et al., 1994). Therefore, three variables (bottom ice algae, phytoplankton, herb copepod) in this model are hypothetical alternative food sources for krill. The availability of these sources is affected by a wide range of environmental conditions. For example, copepod abundance is low in some regions, and therefore, contributes only a small dietary component for krill (Atkinson et al., 2002; Meyer et al., 2009). Uncertain linkages between krill and different variables were used to represent these variabilities (colored lines in Fig. 4.1a). Seven alternative model configurations were tested to investigate the influence of these uncertain interactions to krill responses. These alternative models are described in Appendix C.

The protist model (model B, Fig. 4.1b) illustrates the dietary importance of protists to both krill (Perissinotto et al., 2000; Schmidt et al., 2006; Wickham and Berninger, 2007; Schmidt et al., 2014) and herbivorous/omnivorous copepods (Atkinson, 1995; Wickham and Berninger, 2007). The detritus-protist model (model C, Fig. 4.1c) includes the additional detritus variable to represent the theory that krill ingest detritus (Kawaguchi et al., 1986; Daly, 1990; O'Brien et al., 2011; Schmidt et al., 2011). By resolving krill into two different life stages, lifestage models (model D, Fig. 4.1d; model E, Fig. 4.1e) capture the dietary differences between larval and post-larval krill (reviewed in Meyer, 2012). Sea-ice biota are important for winter survival of larval krill, whereas post-larval krill are usually less dependent on sea ice, and feed on copepods, detritus and protozoans in the water column (Atkinson et al., 2002; Meyer et al., 2002; Quetin and Ross, 2009). The microbial models (Fig. 4.1f, 4.1g, 4.1h) resolve 'protist' into two variables, which represent microbial assemblages in the sea-ice matrix and the underlying water, respectively. Model H only differe from model G by the presence of a positive link from detritus to protist (i).

There is a limited understanding of the coupling between primary production and protozoan biomass. As protozoa graze on both bacteria and algae, the relationship between protozoan and primary production is subject to environmental conditions (Garrison and Mathot, 1996; Garrison et al., 2005; Davidson et al., 2010). Therefore, for each of models B to H, we considered four model structures capturing alternative realities about the relationship between protozoan and primary production (examples are listed in Table 4.1):

(1) Alternative model 1 excludes the linkage between either primary producer (bottom ice algae or phytoplankton) and protists, representing the scenario where protozoa mainly rely on bacterial production, and protozoan biomass is not effected by the availability of primary production (Gowing and Garrison, 1992).

(2) Alternative model 2 includes the interaction between phytoplankton and protists (blue link in Fig. 4.1b to h). Alternative model 2 represents the scenario where phytoplankton is the dominant food source for protozoa (Garrison, 1991a). In models F, G, H detailing protist (w) and protist (i), this link is between phytoplankton and protist (w). Alternative model 2 here represents the scenario where pelagic protozoa graze on phytoplankton, while sea-ice protozoa consume bacteria.

(3) Alternative model 3 has a linkage between bottom ice algae and protists (red link in Fig. 4.1b to h), which represents the scenario where protozoa graze on ice algae (Caron and Gast, 2010). With model F, G, H, this link is between bottom ice algae and protist (i), representing the scenario where sea-ice protozoa consume ice algae, while pelagic protozoan consume bacteria.

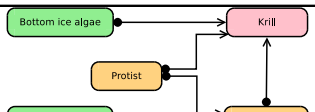
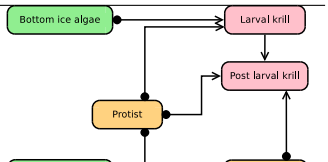
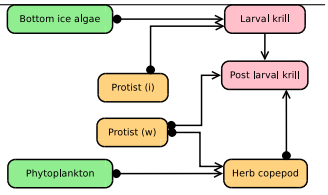
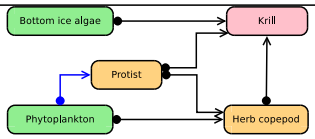
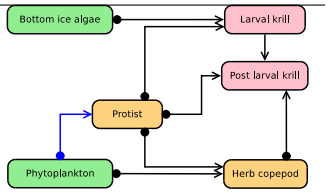
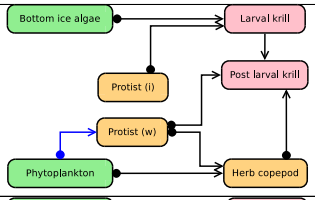
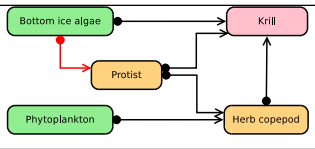
(4) Finally, alternative model 4 includes interactions between both primary producers and protists (both blue and red links in Fig. 4.1b to h). This model configuration represents the scenario where sea-ice protozoa graze on ice algae, and pelagic protozoa graze on phytoplankton (Garrison, 1991a; Gowing and Garrison, 1992; Caron and Gast, 2010).

4.3.3 Perturbation scenario

We applied a perturbation scenario of increased bottom ice algae and decreased phytoplankton to all sets of models. This perturbation simulated a winter condition in sea-ice zones where phytoplankton is scarce in the water column, and sea-ice algae account for the majority of primary production (Arrigo et al., 2010). Winter is a critical season

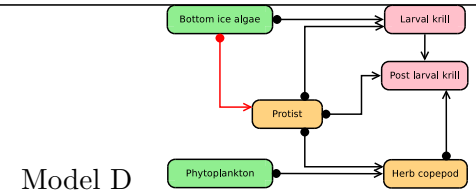
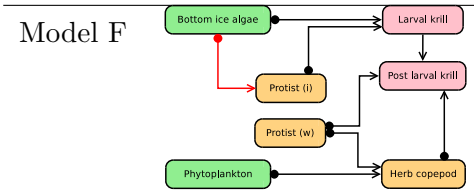
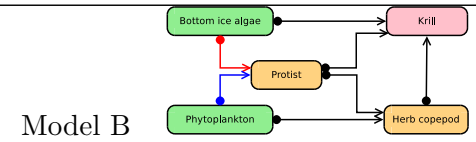
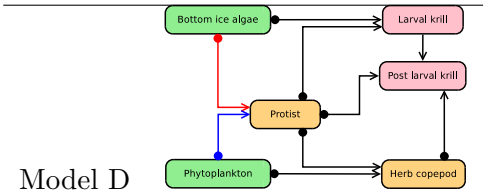
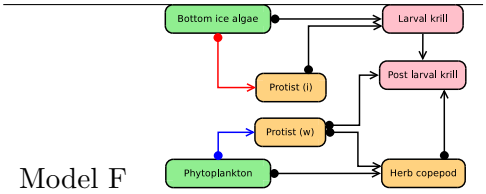
for the survival and recruitment of krill populations, so this perturbation scenario allows us to test how krill populations may respond to winter conditions under different assumptions about their food supplies.

TABLE 4.1: Examples of four model structures representing alternative realities about the relationship between protozoan and primary production. Selected models (models B, D, F) were used here for examples. Detail descriptions are given in "Materials and methods" section. Detail of model configuration is given in Fig. 4.1.

Alternative Model #	Model configuration	Scenarios	References
Alternative Model 1	Model B  Model D  Model F  	Protozoa mainly rely on bacterial production. Protozoan biomass is not affected by the availability of primary production	Gowing and Garrison (1992)
Alternative Model 2	Model B  Model D  Model F  	Protozoa graze on phytoplankton Pelagic protozoa graze on phytoplankton, while sea-ice protozoa consume bacteria	Garrison (1991a)
Alternative Model 3	Model B  	Protozoa graze on ice algae	Caron and Gast (2010)

Continued on next page

Table 4.1 – continued from previous page

Alternative Model #	Model configuration	Scenarios	References
	Model D 		
	Model F 	Sea-ice protozoa consume ice algae, while pelagic protozoan consume bacteria	
Alternative Model 4	Model B 	Sea-ice protozoa graze on ice algae, and pelagic protozoa graze on phytoplankton	Garrison (1991a); Gowing and Garrison (1992); Caron and Gast (2010)
	Model D 		
	Model F 		

4.4 Results

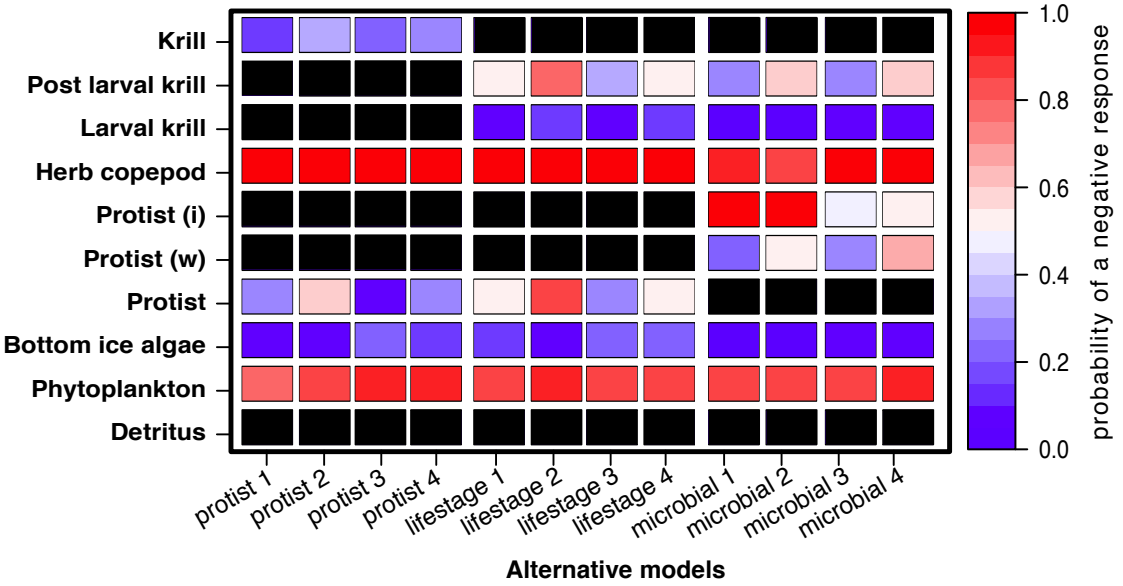
Model outputs are compared among models B, D, F to investigate the effects of increasing model complexity on model responses (Fig. 4.2a). Model B included krill as one variable (referred to as the one-krill model). Models D and F specified krill as larval and post-larval stages, which were referred to as stage-specified models. As shown in Fig. 4.2a, model predictions for krill differed between the one-krill model and stage-specified models. In addition, krill responses from the four alternative models were similar to model B, but varied for models D and F. Model B provided strong support for increases in krill (less than 15% probability of a negative response). Model D and F provided strong support for increases in larval krill (less than 10% probability of a negative

response), but results for post-larval krill varied from moderate support for increases to moderate support for decreases depending on the interaction between protozoa and primary production (ranging from 24% to 69% probability of a negative response).

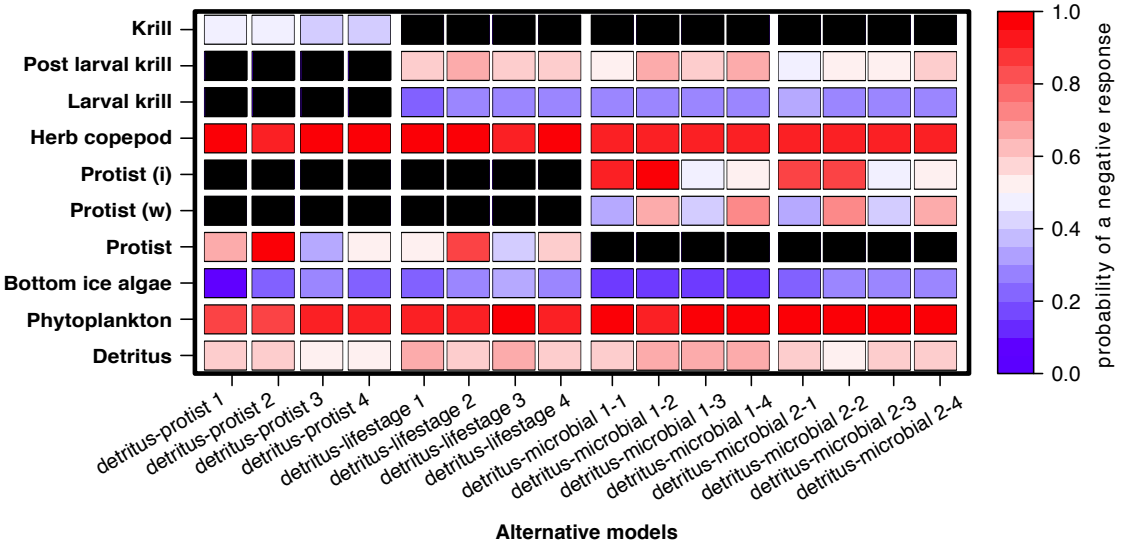
By examining four alternative model configurations, we aimed to identify whether couplings between different primary producers and protists (pelagic and ice-associated) have an effect on krill responses. For model B, similar results among alternative models suggested that links between primary producers and protists had a minor impact on krill responses. However, this effect became significant in terms of the responses of post-larval krill when stage-specified information was included in the model (models D and F). Model D provided moderate support for decreases (69%) in post-larval krill when protist was coupled with pelagic phytoplankton, and moderate support for increases in post-larval krill (34%) when protists were coupled with bottom ice algae. Predictions were ambiguous when both links were included or excluded in the model (52% and 51% probability of a negative response, respectively). Model F further specified protists as sea-ice protists and pelagic protists, and by including the linkage between pelagic protists and phytoplankton, there was a 35% higher probability of decreases for post-larval krill in comparison with alternative structures without this link. In contrast, including the linkage between sea-ice protists and bottom ice algae did not affect the prediction.

Fig. 4.2b shows model outputs from all sets of detritus models (models C, E, G, H) under our simulated winter scenario. As for the case described above, model C was a one-krill model, and models E, G, H were stage-specified models. In comparison with non-detritus models (models B, D, F), detritus models generally predicted a higher probability of decreases in krill. Also, the observed differences among alternative models were eliminated in the detritus models. Model C projected a 35% probability of a negative response for krill, which is nearly 20% higher than that from model B. Models E, G, and H all provided moderate support for increases in larval krill (approximately 20% probability of a negative response), but predictions for post-larval krill were more ambiguous for all alternative models (ranging from 42% to 57% probability of a negative response).

With model B, seven alternative models were constructed by modifying the certainity of model linkages (Appendix C). Fig. 4.3 shows that under a winter scenario (increases in bottom ice algae and decreases in phytoplankton), there was strong support for increases for krill (15% probability of a negative response) when krill were strongly linked with bottom ice algae, and weakly linked with copepods. Strong support for decreases in krill (72% probability of a negative response) only occurred when the link between krill and bottom ice algae was uncertain.



(a) Predicted responses for model B (protist), D (lifestage), and F (microbial)



(b) Predicted responses for model C (detritus-protist), E (detritus-lifestage), G (detritus-microbial 1), H (detritus-microbial 2)

FIGURE 4.2: Predicted responses for model sets under a scenario of increased ice algae and decreased phytoplankton in the water column. For each model, four alternative models are used during the simulation, which is indicated with number 1 to 4. Black indicates variables that are not included in the model. **a**. Predicted responses for model B (protist), D (lifestage), and F (microbial); **b**. Predicted responses for model C (detritus-protist), E (detritus-lifestage), G (detritus-microbial 1), H (detritus-microbial 2).

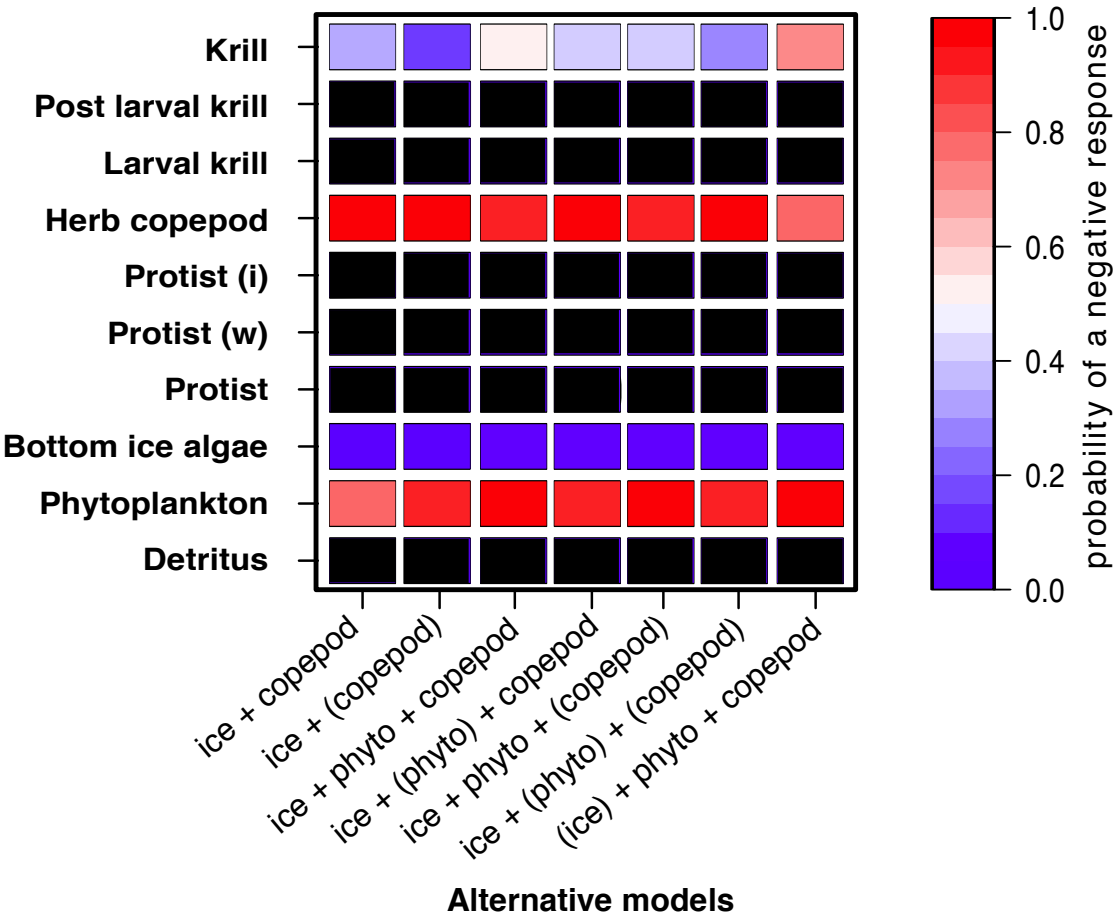


FIGURE 4.3: Predicted responses for the copepod model set under a scenario of increased ice algae and decreased phytoplankton in the water column. ( ) denotes the variable is weakly linked with krill (represented by dash line in the model). Black indicates variables that are not included in the model.

In contrast to the varied responses seen for krill, there was strong support for decreases (higher than 80% probability of a negative response) in herbivorous copepods in all cases (Fig. 4.2, 4.3). When protists were included in the model, there was stronger support for decreases in protists when the link between phytoplankton and protists was included.

4.5 Discussion

Antarctic krill are effective phytoplankton grazers, but are also able to consume other food sources. Food availability is different across krill's major overwintering habitats (Atkinson et al., 2008; Schmidt et al., 2014), and a wide range of feeding theories have been proposed for overwintering krill (Daly, 1990; Quetin and Ross, 1991; Schmidt et al., 2014). This ability of krill to switch diet in winter according to food availability is key to their survival. Our qualitative models explore different possible feeding scenarios,

and model outputs suggest which scenarios support a higher possibility of an increase in krill population.

4.5.1 Feeding on copepods

It is generally accepted that Antarctic krill are able to ingest copepods during winter (e.g. Hopkins and Torres, 1989; Atkinson et al., 2002; Meyer et al., 2009). However, it is still unclear 1) how much the consumption of copepods contributes to krill overwintering diet (e.g. Hopkins and Torres, 1989; Lancraft et al., 1991; Hopkins et al., 1993a; Huntley et al., 1994); and 2) what copepod species krill consume (Huntley et al., 1994; Meyer et al., 2009).

Huntley et al. (1994) suggested a carnivorous diet for overwintering krill. This has been challenged by many studies that only found few copepod remains in the stomach contents of krill (e.g. Meyer et al., 2009; Schmidt et al., 2014). A recent finding suggests that krill constantly feed on a small portion of copepods, and algae are the preferred food whenever possible (Schmidt et al., 2014). Our model, therefore, excluded the idea of krill being completely carnivorous (Huntley et al., 1994). Our copepod model indicated that a strong link between bottom ice algae and krill is the key to ensure a positive prediction for krill, and the certainty of the linkage between copepods and krill has only a minor effect on prediction for krill. Consistent with recent observational studies (Schmidt et al., 2014), our modelling approach suggests that sufficient ice algae is essential for maintaining a krill population during winter, whereas copepod ingestion is beneficial, though not necessary, for krill survival.

Traditionally, only abundant large calanoid copepod species (i.e. *Metridia gerlachei*) were considered to be potential food for krill (Huntley et al., 1994). More recently, highly abundant small copepods (e.g. *Oithona* spp.) were suggested as available food for krill instead of larger species (Atkinson et al., 2002; Meyer et al., 2009). These small copepod species might have a closer relationship with sea ice than large species (Swadling, 2014). Copepods in our models represented species consuming a pelagic phytoplankton diet, therefore, discussion about krill feeding on ice-associated copepods is beyond our model representation. However, it is recommended that future modelling studies need to refine the copepod group into more details to examine the importance of small copepods to krill over the winter.

4.5.2 Feeding on protists

There has been increasing recognition of the significance of protozoa in the diets of both krill and copepods (Meyer et al., 2002; O'Brien et al., 2011; Schmidt et al., 2014). However, simply adding protists into the model did not greatly influence krill responses. The effect of protists only became important when krill were resolved into two life stages in the model (models D and F), and this impact was through the coupling between primary production and protists. When protists were included as one variable in the model (model D), a link between phytoplankton and protists has a negative effect on the model prediction of post-larval krill, and a link between ice algae and protists had a positive effect on the simulated response of post-larval krill. However, when protists are treated as two variables in the model (model E; sea-ice and pelagic protists), the link between phytoplankton and pelagic protozoa retains its negative effect on the model prediction of post-larval krill, but the link between ice algae and sea-ice protists does not have any significant effect on krill responses.

An interesting point here is the discrepancy between model predictions of larval krill and post-larval krill. Although models indicated a high possibility of increases in larval krill, and suggested a strong recruitment in all cases, the post-larval krill population was mainly influenced by protozoa. Although heterotrophic protozoas (i.e. heterotrophic flagellates and ciliates) account for a significant fraction of both pelagic and sea-ice heterotrophic biomass (Garrison and Mathot, 1996; Brierley and Thomas, 2002; Garrison et al., 2005), our understanding regarding of these organisms remains very limited. The dynamics of protozoas are affected by physical, chemical and biological factors (Davidson et al., 2010), and because protozoa graze on both bacteria and algae, the extent of primary production and protozoan production coupling remains unclear (Garrison, 1991b; Brierley and Thomas, 2002). Our results suggested that if krill feed on pelagic protozoas that mainly consume phytoplankton, post-larval krill is likely to experience some degree of decrease. However, if krill feed on protozoans that mainly consume ice algae, bacteria, or both, it is more difficult to predict the changes in post-larval krill populations. We highlight that the relationship between protozoan biomass and primary production will need to be addressed in future studies to understand the dynamics of krill population.

4.5.3 Feeding on detritus

Suspended detrital materials, including zooplankton faecal pellets and particulate organic matter, are a major source of carbon in the ocean, which are contributed by every component in the system (Azam et al., 1993). They suspend in the upper water column in the form of marine snow, and are consumed by filter feeding zooplankton species. On the other hand, adult krill have strong swimming ability and are able to consume benthic detritus (Schmidt et al., 2011).

By including detritus in the model, we expected to see a more positive response in krill. However, predictions were more ambiguous from detritus models (models C, E, G, H) than non-detritus models (models B, D, F). These unexpected results might be explained by the limitation of our method. During our model construction, interactions between detritus and consumers in the model were represented with a positive-positive link (e.g. Fig 4.1e), which assumes the strength of this pairwise relationship is equal. For instance, in model C, the positive-positive link between detritus and krill supposed that the effect of krill grazing on detritus is equivalent to the rate of contribution from krill debris and faeces to detritus. This assumption is difficult to measure, but unlikely in the real world. Considering the relative strength of specific interactions could help to resolve signs of ambiguous predictions (Dambacher et al., 2002, 2007). Unfortunately, we are unable to define the relative strength within this pairwise interaction with current knowledge. To better understand the significance of detrital material in defining krill responses, some questions need to be further investigated in the future. Firstly, although detrital materials have been frequently recognised within krill stomach (Kawaguchi et al., 1986; O'Brien et al., 2011; Schmidt et al., 2011), the actual nutritional contribution of detritus should be evaluated to justify the decision of including this variable into the food web model. Secondly, for each ecological component in the model, we need to be able to estimate the relative amount of excretion into the detrital pool and the amount of detrital material that is utilised. Only by doing so will we be able to define the relative strengths regarding detritus to obtain a more realistic scenario and more accurate predictions.

Our models here are intended to be minimum realistic for the purpose of evaluating how the food web complexity affects krill responses and to identify the critical variables or processes governing krill populations under winter conditions. Models presented here incorporated limited under-ice species interactions. It will be useful to include more zooplankton species into this food web model and to consider formulating alternative models for different seasons of the year. Model A was not a presentation of realistic feeding scenarios since the age separation of krill feeding strategies were determined in field observations (reviewed in Meyer, 2012). However, it represents a commonly accepted

status in the modelling world which considers Antarctic krill as a variable with relatively simple feeding pathways. Finally, model linkages are not directly parameterised, and the model predictions do not capture relative magnitudes of responses due to the nature of qualitative network approach. However, our approach and findings strongly support the need to consider an age-specified resolution for krill in evaluating the krill population dynamics in sea ice zones.

In conclusion, there are several possible feeding scenarios under which a krill population could grow under the sea ice during winter: 1) feeding on sufficient sea ice algae with copepods as a supplementary energy source; 2) feeding on sea-ice algae and sea-ice associated protozoa. Our study has shown the importance of involving stage-specific information during model construction. Finally, we suggest that, in order to better understand krill population dynamics, future studies might usefully target the relationship between primary production and protozoa.

Chapter 5

Conclusion

This study has provided insights into the ontogenetic morphological development of Antarctic krill (*Euphausia superba*), described trophic ecology of the under-ice zooplankton community through stable isotope analysis, and explored optimal feeding scenarios for overwintering krill using qualitative modelling.

5.1 What I learnt

The morphology of Antarctic krill was described in early studies (Fraser, 1936; Marr, 1962), which were fundamental for the establishment of the larval staging system for this species (Kirkwood, 1982). However, these works were all based on field-caught krill samples, and, therefore, provided a record of this species only at certain life stages. My research included five-month observation of Antarctic krill, culminating in a description of their morphological changes through their early life history (Chapter 2). These detailed observations brought new insights into the ontogenetic development of krill and related these morphological changes to function.

The fully developed thoracic legs (referred to as the feeding basket) are the main feeding appendages for adult krill. However, I found during my laboratory observation that thoracic legs can be used for feeding before they become a fully functioning feeding basket. Larval krill are able to perform raptorial feeding as early as in Calyptopis I or II. Under phytoplankton-rich conditions, Calyptopis larvae were able to capture *Artemia* by bending their abdomen inwards, springing towards the prey and grabbing them with their undeveloped thoracic legs. Meyer et al. (2009) has found remains of small copepods in the stomach of larval krill, and my laboratory observation clearly supported their capability to capture small copepods in the environment. A fully functioning feeding basket is

very effective at filtering small particles in the water: ranging from 6.5 μm to 58.2 μm in McClatchie and Boyd (1983), and 1 μm to 30 μm in Kils (1983). Krill are also observed using the feeding basket to scrape ice algae from the bottom of the sea ice (Marschall, 1988). Most krill larvae found in situ during winter are beyond Furcilia III. The feeding basket is not fully developed until Furcilia III. As discussed in chapter 2, Furcilia III is possibly the most energy-demanding stage during the late-larval development. It is possible that krill larvae can develop beyond Furcilia III before the food-depleted season thereby optimising feeding strategies by using their newly developed feeding basket. This might be an adaption for krill to survive through winter.

Krill gradually develop five pairs of pleopods from Furcilia I. The second pair of pleopods produces the strongest propulsion per stroke (Kils, 1983), and is the most important pair for swimming. The second pair of pleopods is fully developed by Furcilia II in general, suggesting a substantial improvement in swimming performance by this stage.

Intermediate larval stages were commonly observed in this study, and they were observed when conditions were optimal (ample supply of food, and no or little competition). This suggested that the intermediate stage is an intrinsic feature during development of larval krill (Feinberg et al., 2006). This makes some larval krill prolong their developmental time through some stages, which enables the coexistence of different stages of larvae. This feature could enhance the opportunity for larval krill to cope with different environmental conditions, and improve the survival rate of this species.

In Chapter 3, I investigated the contribution of sea-ice biota to krill diet using stable isotope analysis. Stable isotope analysis is the only method currently available to distinguish between sea-ice and pelagic food sources. Prior to this investigation, many studies have reported krill aggregations under the sea ice, and suggested that sea-ice biota serves an alternative food source for overwintering krill (e.g. Daly, 2004; Meyer et al., 2009; Quetin and Ross, 2009). However, no direct evidence was provided to verify the importance of sea-ice algae in krill diet (Nicol, 2006). The present study was the first to investigate the contribution of sea-ice biota to krill diet using the stable isotopic method. Stable isotope ratios indicated the feeding plasticity of larval krill (Daly, 2004), as they can feed on sea-ice biota, but also are able to utilise pelagic heterotrophic food sources if sea-ice biota are unavailable. In contrast with larval krill, juvenile and adult krill consume both sea-ice and pelagic biota, but with a preference for a pelagic diet. This might be a reflection of different feeding behaviour between larval, juvenile and adult krill. Larval krill are commonly observed in close contact with sea ice (Marschall, 1988; Daly, 1990; Meyer et al., 2009), but adult krill overwinter in deeper water (Flores et al., 2011). I also extended the analyses and examined a broader range of zooplankton species. Stable isotope profiles indicated that sea-ice biota is an important food source

for several zooplankton taxa, including pteropods, ostracods, small copepods, and amphipods. In Chapter 3 I confirmed the usefulness of stable isotope analyses in studying sea-ice food webs. However, limitations of stable isotope analyses are also apparent, which will be discussed in the Section 5.2.

One of the key goals of understanding under-ice food webs is to apply the acquired knowledge to models and predict responses of this ecosystem to environmental changes. The profound regional differences in the Antarctic sea ice zones make it difficult to produce a single food web model representing the entire sea-ice ecosystem (Atkinson et al., 2008; Stammerjohn et al., 2012; Massom et al., 2013). Even constructing quantitative food web models for a small region requires intensive studies on many ecosystem components (e.g. Ballerini et al., 2014). In addition, the complexity of sea-ice food webs makes it difficult to decide what should be included in a model. Fulton et al. (2003) has suggested the 'minimum realistic' rule where a food web model should be complex enough to represent the real system, but also to be simple enough for clear interpretations. This requires stepwise construction and comparison between models to determine the level of complexity needed. For this purpose, I applied a qualitative modelling approach to discuss the level of information required in a food web model. The results of Chapter 4 suggested the importance of including stage-specific dietary information in model construction. In addition, by using qualitative models to represent different krill feeding scenarios, this approach also highlighted the critical areas for future study. For example, Chapter 4 showed that one of the processes controlling the positive or negative predictions of krill populations in models is the relationship between protozoan and pelagic primary production, which should be investigated in future studies.

5.2 Remaining issues

Morphological description, as applied in this thesis, is important for defining the feeding capability of an animal, but of limited use if not combined with behavioural observations. Knowledge of behaviours helps us understand the function of morphological features. Observing animal behaviour provides valuable knowledge of the mechanisms of the feeding process. Antarctic krill appear as a swarm in the ocean, and their feeding behaviour is affected by these aggregations, however relatively little is currently known about this (Atkinson et al., 2014). Conducting behavioural observations was beyond the scope of this thesis, however, should be investigated in future studies.

One issue remaining unsolved in this thesis is the quantification of the contribution from different food sources to krill diet. This is due to the poor performance of the stable isotope mixing model (SIAR, Parnell et al., 2013). The SIAR model has been successfully

applied for many high-trophic animals (Seymour et al., 2014; Walters et al., 2014). It did not work well here mainly because of the 1) large isotopic variation within each food source, 2) the isotopic overlap between two food sources, and 3) the uncertainty regarding enrichment factors for both stable isotopes. These problems need to be addressed through further investigations. I suggest that the large isotopic variation in each food source is a reflection of a seasonal variation in stable isotope ratios of both sea-ice POM and pelagic POM (Chapter 3). However, more stable isotope studies of sea-ice POM and pelagic POM are needed to be able to determine how their stable isotope ratios change in response to different environmental conditions.

The uncertainty with enrichment factors is possibly related to the turnover rate of stable isotopes. Primary producers have a quick turnover rate and need only days to reflect the isotopic change of their nutrient substrates, whereas zooplankton need weeks to months to reflect the isotopic change of their diet (Frazer et al., 1997; Schmidt et al., 2003; El-Sabaawi et al., 2010). Larval krill take more than eight weeks to reach isotopic equilibrium, and post-larval krill need an even longer period (Frazer et al., 1997; Schmidt et al., 2003). Very few studies have attempted to address the isotopic turnover rates of copepods, and it is unknown if the turnover rates vary across species (El-Sabaawi et al., 2010). Therefore, if the isotopic ratios change in their food sources, it might be reflected to different degrees among species at the time of sampling (Fry and Arnold, 1982; Hobson and Clark, 1992). A better understanding of both enrichment factors and turnover rates requires more laboratory incubation experiments for individual zooplankton species.

Although morphology can tell us what krill are capable of consuming, stable isotope analysis can distinguish if the food is sourced from the sea ice or from the water column, and qualitative models can identify which feeding scenarios are optimal for the maintenance of a krill population. None of the three methods here are able to provide high taxonomic information on krill dietary components. Current available methods that provide high taxonomic dietary information are microscopic analysis and genetic-based approaches. During this work, I also attempted to conduct both microscopic and genetic analysis on krill gut contents to detail dietary information. Unfortunately, neither method achieved this purpose due to the low food intake of the analysed animals (hence results were not included in this thesis). Although unsuccessful with these approaches in my study, these two methods have proven useful in many previous zooplankton feeding studies (e.g. Meyer et al., 2002; Deagle et al., 2010; Jarman et al., 2013; Connell et al., 2014; Grigor et al., 2014), and, therefore, provide complimentary information when used together with stable isotope analyses.

Technical issues regarding sampling

To determine what zooplankton species eat under Antarctic sea ice requires knowledge

of under-ice habitats and sufficient zooplankton samples from different regions. Scuba diving directly under the ice has been the dominant means to observe krill under the ice (Quetin and Ross, 2009). Diving activities requires highly skilled personnel and are restricted by environmental conditions. Underwater vehicles have existed for a while, but only recently has it become possible to operate underwater vehicles under the ice and obtain detailed information and high-resolution footage (e.g. Williams et al., 2015). Sample collection is also a challenge in sea-ice zones. Under-ice zooplankton is concentrated in the surface water just below the sea ice above the depth of 10 m (Flores et al., 2011). However, neither acoustic devices or traditional sampling nets are designed for this depth (Atkinson et al., 2012a). The newly designed Surface and Under Ice Trawl (SUIT, van Franeker et al., 2009) has proven efficient in catching a variety of zooplankton species in the upper 10 m of the under-ice water column (Flores et al., 2011, 2012b, 2014). During the sample collection stages for this thesis, I also used a modified commercial pump to collect zooplankton species (Chapter 3). However, due to the patchiness of zooplankton, the technique of locating zooplankton under the ice remains crucial to ensure quality sample collections.

Both sea-ice conditions and zooplankton ecology show strong seasonal and regional differences around Antarctica. Therefore, to gain a generalised picture of what zooplankton feed on under the sea ice requires an integration of results from different areas in Antarctic sea ice zones. This question cannot be answered by a single sampling trip or a short-term study. In the following section, I discuss the strategies for collaboration.

5.3 For the future

Currently, there are not enough data to produce quantitative estimations of zooplankton dietary components or quantitative modelling outputs that can reliably evaluate future changes. However, better quantification of the contribution of sea-ice biota is the necessary next step to enable more accurate predictions of zooplankton responses to environmental changes.

As discussed above, inadequate representation of species and habitats is a major reason limiting our understanding of zooplankton feeding under the ice. There are not enough samples mainly because 1) it is difficult to access sea-ice zones and zooplankton studies in the sea-ice zones are limited; 2) it is challenging to obtain zooplankton samples in sea-ice zones due to their patchy distribution.

Conducting zooplankton sampling in sea-ice zones requires manpower and is often prohibitively expensive. Recent work, instead of focusing on zooplankton in the sea-ice

zones alone, has compared the whole systems in regions with various types of ice and in adjacent open water (e.g. Quetin and Ross, 2009; Flores et al., 2011, 2012b, 2014). In comparison with ice-covered regions, the ice-associated open water system provides a "control", which has areas of both unusual seasonality and climatic variability. Feeding ecology under the sea ice is a complex matter. Zooplankton may obtain food from the under-ice water column, but this system is intricately associated with the sea ice. For example, sea-ice biota seed the underlying waters when sea ice starts melting, and therefore, affect the primary production in the water column (Riaux-Gobin et al., 2011). Additionally, nutrients released from the sea ice are likely to enhance the growth of phytoplankton in the water column (Lannuzel et al., 2014, 2015).

Clearly, it is beyond the ability of one research team to study all regions and seasons. Comparing the whole system (i.e. abundance, diversity, feeding, food sources, condition indices, etc.) between regions brings another aspect on zooplankton feeding studies. Future studies on sea ice and ice-associated zooplankton require collaborative efforts between different research groups and different nations. International collaboration would bring experts together and optimise the use of resources. One important issue that requires attention is the careful planning of projects. Modelling tools are useful to integrate information and identify key questions for future empirical studies. These tools should be utilised during the process of developing a project plan.

Another critical issue is to apply standardised methods so that it is possible to compare results between studies. For example, although the stable isotope approach has been applied to study zooplankton trophic relationships by many researchers, no standardised method has been developed in terms of sample preservation and sample treatment. It is unknown how comparable results are from different methods. Research related to this issue should be a high priority because it is important to establish a baseline for cross-study comparison.

Regarding the difficulty in obtaining zooplankton samples, the SUIT seems to be able to overcome this by trawling large areas under the ice. However, if using other sampling instruments, good locating devices are important during sampling operations. For example, underwater vehicles with mounted cameras can potentially serve as locating devices, which can transfer under-ice images back to operations instantaneously so as to direct the sampling device. Furthermore, images and footage obtained by underwater vehicles provide valuable information on under-ice habitats and the in situ feeding and behavioural record of different zooplankton species, which is crucial for our further understanding of their feeding ecology.

In conclusion, Antarctic sea ice has undergone rapid changes in many areas over the past few decades. In order to understand the ecological implication of these changes,

knowledge regarding the diet of Antarctic krill and other zooplankton species in sea-ice zones is essential. The fundamental goal is to understand the role of sea ice in this whole system. Sea ice not only provides direct food sources for zooplankton, but it also influences the production in the water column. Therefore, it is worthwhile to compare the systematic differences between sea-ice covered regions with associated open water regions. Future studies regarding zooplankton under-ice feeding requires the design of collaborative projects and the utilisation of advanced technologies. This holistic approach will enable a better understanding of zooplankton in circumpolar under-ice habitats.

Appendix A

Supporting measurements during Sea Ice Physics and Ecosystems eXperiment (SIPEX) and SIPEX-2.

TABLE A.1: Ice station (Stn), sampling date (Date, UTC), latitude (Lat), longitude (Lon), air temperature (Air temp), surface water temperature (Sst), water salinity, mean ice thickness (Ice), mean snow depth (Snow), ice bulk salinity (range), ice temperature (Ice temp, mean and range), brine volume fraction (Vb/V, mean and range), Chlorophyll *a* (Chl *a*) concentration of bottom 0.10 m of ice core (Bottom Chl *a*), Chl *a* integrated over the entire ice thickness (Integrated Chl *a*), calculated % of integrated Chl *a* in bottom 0.10 m ice, Under-ice water Chl *a* for the sea-ice stations during SIPEX (2007) and SIPEX-2 (2012). Nd = not determined.

Stn	Date	Lat (S)	Lon (E)	Air temp (°C)	Sst (°C)	Water salinity	Ice (m)	Snow (m)	Ice salinity	Ice temp (°C)	Vb/V (%)	Bottom Chl *a* (μg L^{-1})	Integrated Chl *a* (mg m^{-2})	Bottom %	Water Chl *a* (μg L^{-1})
SIPEX (2007)															
1	11/09/07	64°14'	128°00'	-17.87	-1.84	34.104	0.59	0.05	5.0-11.4	-5.7 (-9.7 to - 2.3)	7.8 (5.2 - 13.2)	3.96	0.54	62.18	0.08
2	12/09/07	64°29'	128°05'	-21.35	-1.83	34.179	0.96	0.04	4.5 to 12.1	-6.6 (-9.6 to -2.2)	7.2 (3.3 to 17.7)	5.56	0.76	41.51	0.08
3	14/09/07	64°24'	127°11'	-19.49	-1.86	34.201	0.52	- 0.04	5.5 to 18.7	-7.2 (-11.7 to -2.7)	8.4 (4.4 to 16.7)	3.12	0.32	80.52	0.10
5	18/09/07	65°31'	124°45'	-20.94	-1.86	34.278	0.90	0.01	5.2 to 14.2	-6.4 (-10.2 to -2.0)	7.5 (3.9 to 21.6)	3.89	0.42	78.79	0.05

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Table A.1 – continued from previous page

Stn	Date	Lat (S)	Lon (E)	Air temp (°C)	Sst (°C)	Water salinity	Ice (m)	Snow (m)	Ice salinity	Ice temp (°C)	Vb/V (%)	Bottom Chl a (μg L^{-1})	Integrated Chl a (mg m^{-2})	Bottom %	Water Chl a (μg L^{-1})
6	21/09/07	65°35'	122°35'	-14.06	-1.86	34.007	0.83	0.01	6.3 to 16.4	-5.3 (-9.1 to -2.0)	10.0 (5.1 to 20.1)	0.54	0.08	52.38	0.03
7	22/09/07	65°34'	121°31'	-13.37	-1.86	34.210	0.55	0.03	7.5 to 12.6	-5.1 (-7.2 to -2.8)	11.2 (5.7 to 20.1)	25.55	2.38	91.19	nd
8	25/09/07	65°33'	118°52'	-7.47	-1.85	34.229	0.39	0.01	7.2 to 16.0	-3.7 (-4.8 to -2.4)	13.1 (8.0 to 17.4)	1.30	0.11	92.95	0.01
9	28/09/07	65°21'	118°34'	-13.38	-1.73	34.353	0.97	0.05	3.4 to 9.0	-3.3 (-5.1 to -1.7)	9.2 (4.6 to 34.8)	43.16	5.81	59.56	0.01
10	30/09/07	64°57'	119°08'	-16.33	-1.84	34.381	1.32	0.00	3.4 to 9.8	-4.4 (-6.5 to -2.1)	8.0 (2.7 to 19.6)	18.41	2.39	65.85	0.06

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Table A.1 – continued from previous page

Stn	Date	Lat (S)	Lon (E)	Air temp (°C)	Sst (°C)	Water salinity	Ice (m)	Snow (m)	Ice salinity	Ice temp (°C)	Vb/V (%)	Bottom Chl a (μg L^{-1})	Integrated Chl a (mg m^{-2})	Bottom %	Water Chl a (μg L^{-1})
11	3/10/07	65°01'	117°32'	-7.13	-1.86	34.290	1.08	0.09	2.1 to 8.1	-4.5 (-6.8 to -2.0)	7.2 (3.1 to 18.9)	33.16	3.28	84.96	0.03
12	5/10/07	64°53'	116°58'	-8.58	-1.84	34.301	1.99	0.09	3.6 to 14.1	-4.8 (-7.0 to -2.0)	8.4 (3.2 to 16.6)	35.62	13.61	12.55	nd
13	6/10/07	64°44'	116°49'	-11.23	-1.85	34.352	0.82	0.05	4.5 to 12.3	-3.6 (-5.3 to -2.0)	10.8 (4.8 to 24.2)	74.83	8.43	76.79	0.05
14	7/10/07	64°19'	116°49'	-11.12	-1.85	34.405	0.58	0.07	5.5 to 17.8	-3.2 (-5.5 to -2.1)	12.3 (8.3 to 16.2)	7.57	1.23	56.37	0.04
15	10/10/07	64°41'	120°37'	-6.50	-1.70	33.838	0.48	0.05	4.0 to 11.2	-2.6 (-3.4 to -2.0)	11.6 (7.2 to 13.9)	3.70	0.75	20.38	nd
SIPEX II 2012															

Continued on next page

Table A.1 – continued from previous page

Stn	Date	Lat (S)	Lon (E)	Air temp (°C)	Sst (°C)	Water salinity	Ice (m)	Snow (m)	Ice salinity	Ice temp (°C)	Vb/V (%)	Bottom Chl a (μg L^{-1})	Integrated Chl a (mg m^{-2})	Bottom %	Water Chl a (μg L^{-1})
2	27/09/12	64°23'	120°05'	-19.40	-1.80	34.267	0.75	0.08	3.8 to 7.4	-4.3 (-6.7 to -2.3)	7.8 (5.8 to 12.5)	1.62	1.06	22.31	0.16
3	3/10/12	64°53'	120°53'	-21.71	-1.83	34.247	2.00	1	4.3 to 9.7	-2.1 (-3.5 to -1.6)	14.9 (10.3 to 21.1)	0.51	0.41	21.15	0.11
4	6/10/12	65°08'	121°02'	-13.82	-1.83	34.207	1.05	0.60	4.0 to 8.0	-2.2 (-2.9 to -1.6)	13.8 (9.5 to 22.9)	0.16	2.82	1.28	0.10
6	12/10/12	65°16'	120°00'	-8.96	-1.86	34.238	1.40	0.10	4.0 to 12.5	-1.9 (-2.1 to -1.6)	18.7 (10.8 to 33.7)	13.46	3.80	83.78	0.25
7	22/10/12	65°16'	118°58'	-7.45	-1.87	34.238	0.88	nd	3.4 to 11.1	-2.0 (-2.2 to -1.6)	15.4 (8.2 to 31.8)	7.94	5.18	32.73	nd

Continued on next page

Table A.1 – continued from previous page

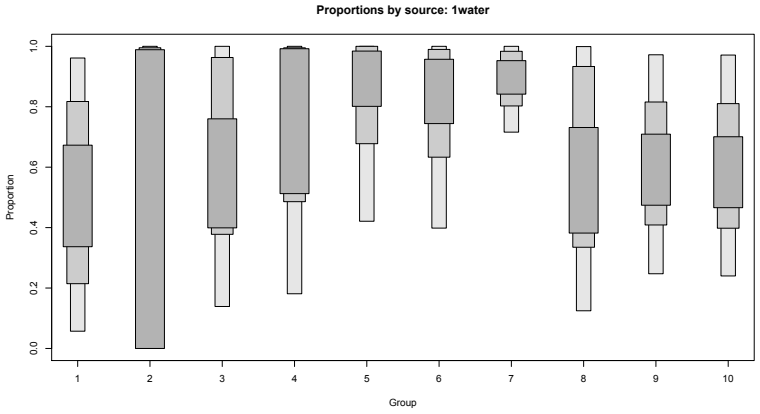
Stn	Date	Lat (S)	Lon (E)	Air temp (°C)	Sst (°C)	Water salinity	Ice (m)	Snow (m)	Ice salinity	Ice temp (°C)	Vb/V (%)	Bottom Chl a (μg L^{-1})	Integrated Chl a (mg m^{-2})	Bottom %	Water Chl a (μg L^{-1})
8	27/10/12	64°52'	116°49'	-8.50	-1.86	34.236	2.18	0.50	3.5 to 13.4	-1.8 (-2.3 to -1.2)	19.1 (10.0 to 36.1)	0.91	2.91	5.31	nd

Appendix B

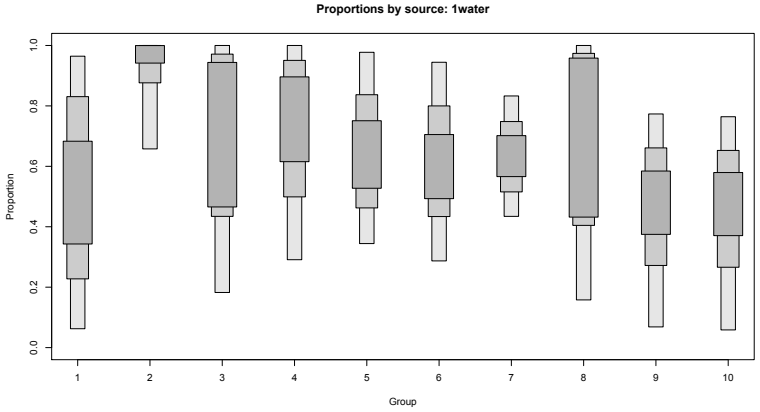
Stable isotope mixing model (SIAR) outcomes.

TABLE B.1: SIAR model predicted estimates of contribution from water particulate organic matter (POM) and sea-ice POM in different zooplankton taxa collected during late winter/early spring off East Antarctica during SIPEX (2007). Values are means (95% interval).

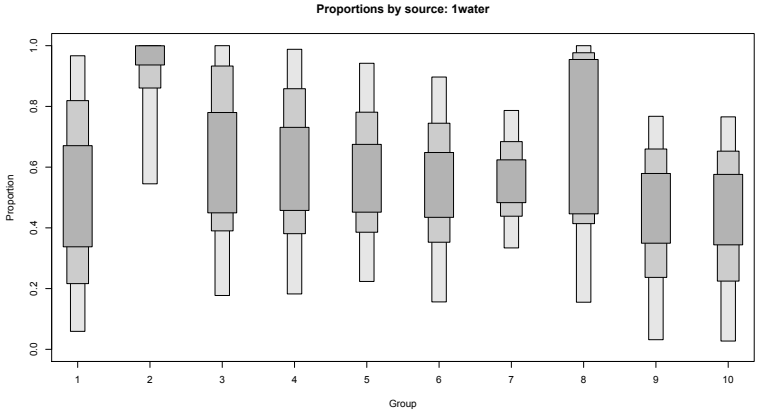
Species	Water POM (%)	Sea-ice POM (%)
Calanus propinquus	88 (55 to 100)	12 (0 to 45)
Euchaeta antarctica	60 (17 to 100)	40 (0 to 83)
Euphausia superba adult	58 (19 to 99)	42 (1.2 to 81)
Euphausia superba juvenile	57 (22 to 94)	43 (5.9 to 78)
Euphausia superba furcilia	57 (22 to 94)	47 (10 to 84)
Thysanoessa macrura	56 (33 to 78)	44 (22 to 67)
Sagitta marri	60 (15 to 100)	40 (0 to 85)
Limacina helicina	44 (3 to 77)	56 (23 to 97)
Primno macropa	44 (3.3 to 77)	56 (23 to 97)



(a)



(b)



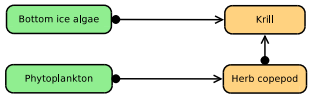
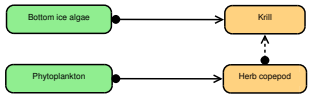
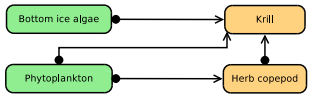
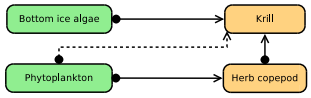
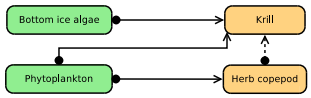
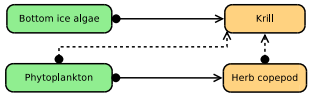
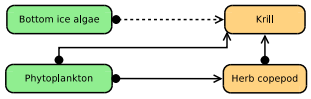
(c)

FIGURE B.1: SIAR model predicted estimates of dietary proportion from water particulate organic matter (POM) in different zooplankton taxa collected during late winter/early spring off East Antarctica during SIPEX (2007). Enrichment factors for δ^{13}C and δ^{15}N are **a**. 3.4 ± 1‰ and 1.5‰; **b**. 2.2 ± 0.3‰ and 0.5 ± 0.13‰; and **c**. 3.4 ± 1‰ and 0.4 ± 1.3‰. Groups of zooplankton: 1 = *Calanoides acutus*, 2 = *Calanus propinquus*, 3 = *Euchaeta antarctica*, 4 = *Euphausia superba* adult, 5 = *Euphausia superba* juvenile, 6 = *Euphausia superba* furcilia, 7 = *Thysanoessa macrura*, 8 = *Sagitta marri*, 9 = *Limacina helicina*, 10 = *Primno macropa*.

Appendix C

Alternative model structures for the copepod model and ecological interactions represented in these models.

TABLE C.1: Ecological interactions represented in copepod models.

Model	Description	Links (refer to Fig 4.1a)	References	Notes
	Antarctic krill consume ice algae and copepod	Green, Blue	Hopkins and Torres (1989); Hopkins et al. (1993b); Huntley et al. (1994)	
	Ice algae is important food item, and copepod is rarely consumed	Green, Blue (weak)	Atkinson et al. (2002); Meyer et al. (2009)	Small amount of copepod remaining is found in krill stomach content
	Ice algae, phytoplankton and copepod all contribute to diet of krill	Green, Blue, Red		The scenario where all three food sources contribute to the diet of krill equally
	Ice algae and copepod are major food item, phytoplankton is secondary food item	Green, Blue, Red (weak)		Phytoplankton is scarce in the water column, and cannot be a major food item
	Ice algae and phytoplankton are major food item, copepod is secondary food item	Green, Blue (weak), Red		Krill is mainly herbivorous, consume little copepod
	Ice algae is the major food item, both phytoplankton and copepod are rarely consumed	Green, Blue (weak), Red (weak)		
	Both phytoplankton and copepod are major food items, and ice algae is rarely consumed	Green (weak), Blue , Red		Ice algae is not available in the region

Appendix D

Ecological interactions represented in models B to H.

TABLE D.1: Ecological interactions represented in models B to H.

From	To	Weight	References	Notes
Model B, C, D				
Bottom ice algae	Krill	1	Daly (1990); Quetin and Ross (2009)	
Krill	Bottom ice algae	-1	Daly (1990); Quetin and Ross (2009)	
Herb Copepod	Krill	1	Atkinson et al. (2002); Meyer et al. (2009); Schmidt et al. (2014)	
Krill	Herb Copepod	-1	Atkinson et al. (2002); Meyer et al. (2009); Schmidt et al. (2014)	
Protist	Krill	1	Meyer et al. (2009); Schmidt et al. (2014)	
Krill	Protist	-1	Meyer et al. (2009); Schmidt et al. (2014)	
Phytoplankton	Herb Copepod	1	Jia et al., submitted (Chapter 3 of this thesis)	

Continued on next page

Table D.1 – continued from previous page

From	To	Weight	References	Notes
Herb Copepod	Phytoplankton	1	Jia et al., submitted (Chapter 3 of this thesis)	
Protist	Herb Copepod	1	Atkinson (1995)	
Herb Copepod	Protist	-1	Atkinson (1995)	
Larval krill	Post-larval krill	1		Krill growth and recruitment
Protist	Larval krill	1	Daly (2004); O'Brien et al. (2011); Meyer (2012)	
Larval krill	Protist	-1	Daly (2004); O'Brien et al. (2011); Meyer (2012)	
Protist (i)	Larval krill	1	Daly (1990); Meyer et al. (2009)	
Larval krill	Protist (i)	-1	Daly (1990); Meyer et al. (2009)	
Model E, F, G, H - detritus model				
Bottom ice algae	Detritus	1	Kiørboe (2001)	
Phytoplankton	Detritus	1	Kiørboe (2001)	
Protist	Detritus	1	Kiørboe (2001)	
Detritus	Protist	-1	Azam et al. (1993)	
Krill	Detritus	1	Kiørboe (2001); Atkinson et al. (2012b)	
Detritus	Krill	-1	O'Brien et al. (2011)	
Herb Copepod	Detritus	1	Kiørboe (2001)	
Detritus	Herb Copepod	-1	Hopkins et al. (1993a,b)	
Alternative model linkages				
Bottom ice algae	Protist (Protist(i))	1	Arrigo et al. (2010); Caron and Gast (2010)	

Continued on next page

Table D.1 – continued from previous page

From	To	Weight	References	Notes
Protist (Protist(i))	Bottom ice algae	-1	Arrigo et al. (2010); Caron and Gast (2010)	
Phytoplankton	Protist (Protist(w))	1	Buitenhuis et al. (2010)	
Protist (Protist(w))	Phytoplankton	-1	Buitenhuis et al. (2010)	

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